GIBBERELLIC ACID: TRANSLOCATION, METABOLISM AND EFFECTS ON PEEL QUALITY OF 'MARSH' GRAPEFRUIT (*Citrus paradisi* MACF.)

By

Louise Ferguson

A DISSERTATION PRESENTED TO THE GRADUATE SCHOOL
OF THE UNIVERSITY OF FLORIDA IN
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August 1984

Chairman: Dr. F. S. Davies
Major Department: Horticultural Science

Gibberellic acid maintains 'Marsh' grapefruit (Citrus paradisi Macf.) peel quality by delaying senescent peel color development and loss of peel firmness. Combined with 2,4-dichlorophenoxyacetic acid (2,4-D), which prevents preharvest fruit drop, the two extend the grapefruit harvest season. It was not known if decreased irrigation affects this treatment. It also was not known if postharvest application of this treatment would produce the same effects. In addition, GA_3 uptake, translocation and metabolism had not been examined. This dissertation was undertaken to answer these questions.

'Marsh' grapefruit on rough lemon (Citrus jambhiri Lush.) rootstock received irrigated and unirrigated treatments. Half of each irrigation treatment received a GA_3 and 2,4-D spray at colorbreak. Preharvest sprays of GA_3 and 2,4-D extended the grapefruit harvest season by increasing fruit removal and rind puncture force, delaying development of senescent color, and decreasing late-season and

postfreeze fruit drop. Although soil moisture content in the top 0.9 m of unirrigated blocks was reduced by approximately 40%, leaf water potentials of these trees and performance of GA_3 and 2,4-D were unaffected.

Experiments were carried out comparing the effects of a preharvest GA_3 and 2,4-D spray, a postharvest GA_3 and 2,4-D dip, and combined spray and dip treatments on peel quality and decay during storage. Fruit harvested in January, March, and May were stored for 12 weeks at 15.5°C. The GA_3 and 2,4-D treated fruit had less senescent color development, loss of puncture strength, and decay than controls. The three treatments were equally effective for January and March harvests, while preharvest and combined treatments were more effective than a postharvest treatment in May.

Applied ^{14}C-GA_3 was absorbed by peels and leaves of attached fruit within 1 hour, translocated from leaves to peel and the reverse within 4 to 8 hours, and persisted in leaves and peel in measurable amounts for 8 weeks. Accumulation was higher in peels than in leaves no matter where the ^{14}C-GA_3 was applied. Approximately half the absorbed ^{14}C-GA_3 remained in the applied form and approximately half was converted to a water-soluble form within 96 hours. Recovery was less, but metabolism was similar, when ^{14}C-GA_3 was applied to detached fruit.

CHAPTER I

GIBBERELLIC ACID IN GRAPEFRUIT

Gibberellic acid (GA_3) effectively delays normal peel senescence of citrus (16,37,59,63,70,71). The combination of GA_3 with 2,4-dichlorophenoxyacetic acid (2,4-D), which delays preharvest fruit drop (46,99) and deterioration in storage (89), provides a means of extending the grapefruit harvest season (2,25,35) and postharvest storage life (1).

Inconsistent treatment effects may result from a number of environmental factors, including temperature and tree water status. Insufficient irrigation (35), or heavy precipitation late in the harvest season (F.S. Davies and M.A. Ismail, personal communication, 1979) has been observed to decrease the efficacy of GA_3 and 2,4-D in preventing fruit drop and maintaining peel quality. The experimental label for Pro-Gibb®, a commercial GA_3 formulation, currently advises that results may vary depending on environmental conditions. The first objective of this dissertation was to determine if a reduction in soil moisture content following GA_3 and 2,4-D application reduced the efficacy of these materials in extending the harvest season.

Preharvest sprays of GA_3 and 2,4-D decrease storage losses of grapefruit (1). Postharvest dips of GA_3 have produced similar results with 'Shamouti' oranges (37). Postharvest 2,4-D dips also maintain peel quality and decrease decay of grapefruit in storage (89,97). Combining GA_3 and 2,4-D preharvest or postharvest does not alter their efficacy

(2,25,35). The cost of application is lower postharvest than preharvest; however, GA_3 and 2,4-D are not registered for postharvest use on grapefruit. The second objective of this dissertation was to determine whether a preharvest or postharvest application of GA_3 and 2,4-D was better for maintaining grapefruit quality in storage.

Applications of ^{14}C-GA_3 to 'Shamouti' orange peel indicate that it is taken up by flavedo, persists there in diminishing amounts for 100 days, and appears to remain in the applied form (38,39). Beyond this, little else is known about the fate of ^{14}C-GA_3 applied to citrus peel. The third objective of this dissertation was to characterize uptake, translocation, persistence, and metabolism of ^{14}C-GA_3 by grapefruit leaves and fruit.

Determining the first two objectives (the effects of decreased soil moisture content on the efficacy of preharvest applications of GA_3 and 2,4-D in extending the harvest season, and the better time to apply GA_3 and 2,4-D for extending storage life) will enable more economical use of GA_3. Determination of the third objective, the fate of ^{14}C-GA_3 applied to grapefruit peel and leaves, will provide some fundamental physiological evidence for the observed effects of GA_3 on grapefruit peel quality.

CHAPTER II

GROWTH REGULATOR AND LOW-VOLUME IRRIGATION EFFECTS ON GRAPEFRUIT QUALITY AND FRUIT DROP

Introduction

The grapefruit harvest season has been extended in South Africa (35), Central Florida (2), and Australia (25) by applying dilute sprays of GA_3 and 2,4-D at colorbreak. The GA_3 delayed peel color development (2,25,35,59) and loss of peel firmness (2,25), while 2,4-D decreased the characteristic late-season fruit drop (2,25,35,46). The combination maintained acceptable peel quality as late as June under Florida conditions (2). However, not all GA_3 and 2,4-D sprays have produced consistent results. These inconsistent treatment effects may be the result of environmental factors, including temperature and tree water status.

Literature Review

Effects of GA_3 and 2,4-D on External Quality of Grapefruit

The external quality of grapefruit is determined by color, firmness, and presence of blemishes. As peel senesces, it progresses from pale yellow to orange-yellow due to chlorophyll loss (18), is easily punctured and deformed due to loss of firmness, and is more susceptible to decay pathogens. Sprays of GA_3 and 2,4-D applied at the time of colorbreak, mid-November to December, delay orange-yellow color development (2,18,25,35,59,61), probably by retarding chloroplast to

chromoplast conversion in citrus peel (18,43,105). Sprays of GA_3 applied when grapefruit are less than 10 mm in size and at higher concentrations than the usual 10-25 ppm GA_3 (17) cause regreening of grapefruit, as do nitrogen nutritional treatments (39). However, regreening is usually not a problem with grapefruit (18). Sprays of GA_3 and 2,4-D also delay loss of peel firmness (2,25). Applied alone, GA_3 produces a more compact albedo (16,18,69,71) and decreases peel blemishes (16,71). Alone, 2,4-D decreases corky spot of grapefruit if applied within 2 months of anthesis (40). These effects are enhanced by inclusion of potassium or ammonium nitrate in preharvest sprays (4,28,71), possibly through enhancement of endogenous GA_3 production (69). (See Appendix I.)

Effects of GA_3 and 2,4-D on Internal Quality of Grapefruit

Preharvest colorbreak sprays of GA_3 and 2,4-D have not been demonstrated to produce consistent, significant changes in internal quality of grapefruit. Grapefruit internal quality is determined by total soluble solids (TSS), percentage of acid, TSS/acid ratio, and percentage of juice. Reports of the effects of colorbreak sprays of GA_3 and 2,4-D on these indices are conflicting and inconsistent (2,16,17,25,35, 59,99,100). The most frequent reports are of a slight increase in the percentage of juice and a slight delay in the decrease in the percentage of acid late in the season (15). The general consensus is that differences, if created, are slight. Another index of grapefruit internal quality is seed sprouting, which occurs after March and renders fruit unmarketable. Ali Dinar et al. (2) reported that colorbreak sprays of GA_3, 2,4-D, and the combination decreased seed sprouting. However,

Albrigo et al. (1) were unable to corroborate this finding. A last index of internal grapefruit quality is granulation, which occurs late in the harvest season and increases with time. There are no reports that GA_3 and 2,4-D decrease this problem.

Effects of GA_3 and 2,4-D on Fruit Drop

One of the first uses of growth regulators was to prevent preharvest fruit drop of grapefruit (100). Numerous reports indicate that colorbreak sprays of 2,4-D at 8-20 ppm delayed late-season grapefruit drop (2,16,25,32,35,69). In contrast, Kokkalos (59) did not reduce late-season fruit drop in 2 of 4 years by using 2,4-D. Explant work has shown that 2,4-D delays abscission by delaying the rise of cellulases and polygalacturonases in the abscission zone, thereby delaying separation of calyx from fruit (41). Goldschmidt (69) suggested that 2,4-D is an antagonist of ethylene. Alone, GA_3 has never been demonstrated to decrease fruit drop.

Effects of Decreased Irrigation on the Efficacy of GA_3 and 2,4-D

Gilfillan et al. (35) observed that insufficient irrigation late in the harvest season decreased the efficacy of GA_3 and 2,4-D in preventing fruit drop and maintaining peel quality. F.S. Davies and M.A. Ismail (personal communication, 1979) observed no effect of GA_3 and 2,4-D on peel firmness during a May and June when precipitation was higher than average. Kokkalos (53) reported GA_3 and 2,4-D to be ineffective in preventing fruit drop in Cyprus. The label for Pro-Gibb®, a commercial GA_3 product, currently advises, "Results may vary . . . depending on environmental conditions."

The objective of this experiment was to determine if a reduction in soil moisture following GA_3 and 2,4-D application reduces the efficacy of these regulators in extending the harvest season of 'Marsh' grapefruit under Florida conditions.

Materials and Methods

Two 210-tree blocks of 35- to 40-year-old 'Marsh' grapefruit (Citrus paradisi Macf.) trees on rough lemon (Citrus jambhiri Lush.) rootstock from a grove located near Lake Alfred, Florida, were used sequentially during the 1980-81 and 1981-82 seasons. Trees were hedged north-south, spacing was 4.5×9.1 m, and soil type was Astatula fine sand. Microsprinklers delivering 80 liters per hour were located between alternate trees. Fertilizer, pesticide, and irrigation practices were consistent among treatments and typical of groves in Central Florida.

Forty completely randomized single-tree plots and 20 completely randomized two-tree plots were used during 1980-81 and 1981-82, respectively. Microsprinklers of 20 of the single-tree plots and 10 of the two-tree plots as well as those of immediately surrounding trees were capped 1 week prior to GA_3 and 2,4-D application. Half the number of irrigated and unirrigated trees were sprayed after colorbreak on November 12, 1980, and December 14, 1981, with 50 liters of an aqueous solution of 20 mg per liter each GA_3 and 2,4-D and 0.025% Triton X-77. The four treatments, irrigated sprayed and unsprayed and unirrigated sprayed and unsprayed, were analyzed as a 2×2 factorial experiment.

The soil moisture content (SMC) was determined using a Troxler neutron scattering device (Pacheco, CA). Three measurements were made monthly at 0.45- and 0.90-m depths for one location per tree and averaged to yield the SMC of the top 0.90 m of soil. Leaf water potentials (ψ_L) of six randomly selected leaves from equally spaced canopy positions of each tree were determined using a pressure chamber (91). Leaves remained on damp paper towels in sealed plastic bags in an ice chest until immediately before measurement. Leaf samples were collected at 4 AM and 11 AM on the same day that SMC measurements were taken. Sampling times were selected to reflect periods of minimum and maximum water stress, respectively (102).

Fruit drop was determined monthly by counting and removing all fruit within the dripline. Twenty randomly selected fruit per tree were collected monthly to determine fruit removal force (FRF), peel color, rind puncture force (RPF), and internal quality. The FRF was determined by an Ametek hand pull tester (Landsdale, PA), peel color by a Hunter Color Difference Meter (Fairfax, VA), and RPF by an Instron penetrometer with a 0.63-cm radius flat head moving at 20 cm per minute (Canton, MA). The Hunter Color Difference Meter measures chromaticity dimensions of colors and expresses them as "a/b" ratios; "a" measures redness when positive and greenness when negative; "b" measures yellowness when positive and blueness when negative. The ratio indicates proportions of the above colors. A pale yellow grapefruit typically has an "a/b" ratio ranging from -0.1 to 0.2. Internal quality was evaluated by automated systems analysis giving total soluble solids (TSS) by the specific gravity method, percentage of acid, TSS/acid ratio, percentage of juice, and weight per fruit including peel

(23). An additional sample of 20 fruit per tree was collected to determine the extent of seed sprouting. 'Marsh' grapefruit trees in this study bore an average of 1500 fruit. The gradual loss of 350 fruit to sampling and 180 fruit to drop over 6 months should not have affected the quality of the remaining fruit (79).

Results and Discussion

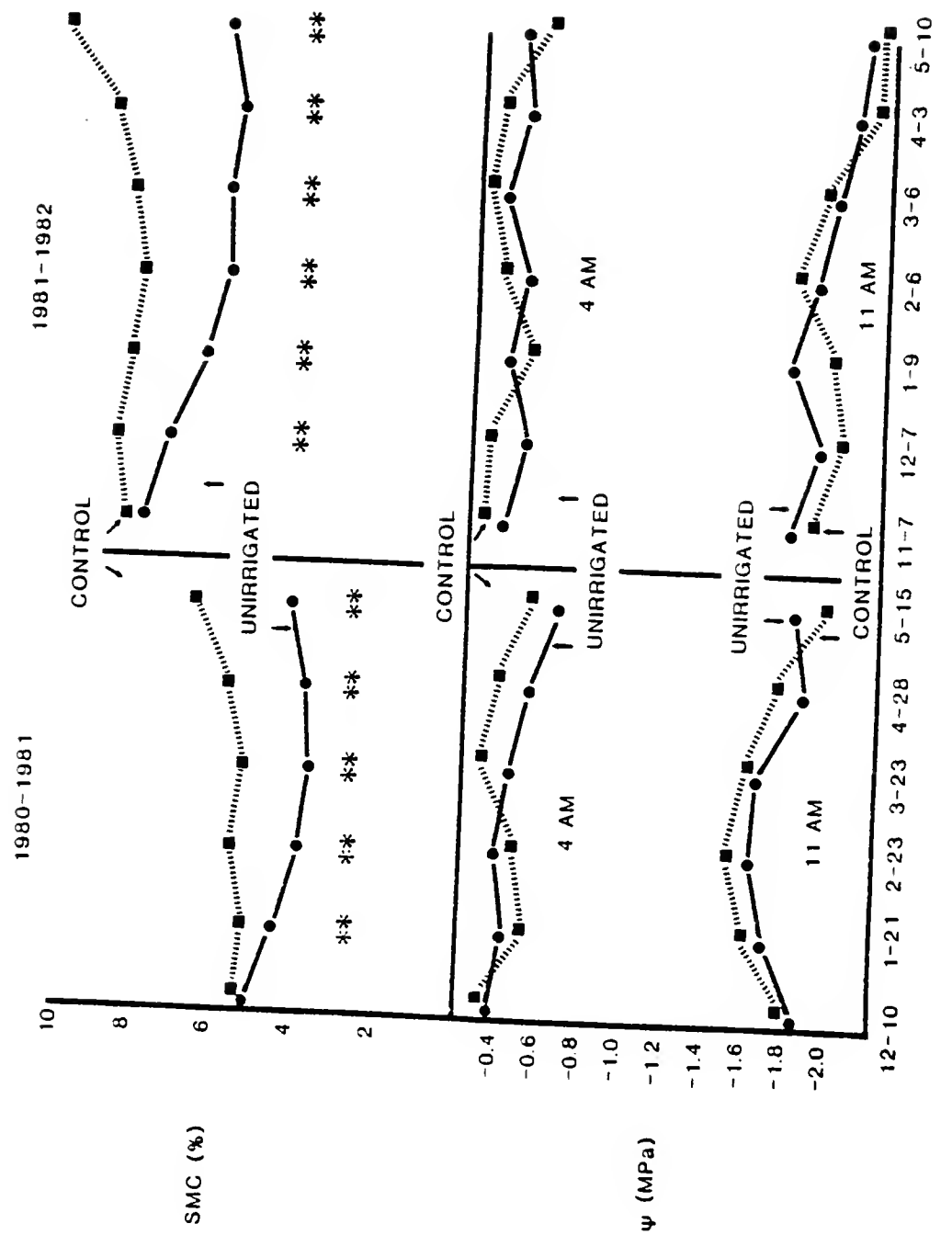
Effects of SMC on Ψ_L

The SMC was significantly lowered in the top 0.90 m of soil in all unirrigated plots on all sampling dates in both seasons; yet the ψ_L values of irrigated and unirrigated trees were comparable at 4 AM and 11 AM (Fig. 2-1). Davies et al. (21) reported a similar lack of correlation between ψ_L value and SMC for 'Orlando' tangelo trees in the field. They hypothesized that tree capacitance and ability to control water loss via stomatal closure are factors that affect the reliability of SMC as an indicator of a tree's water status. Their results and those given here are consistent with grower observations that large, well-established trees, particularly those on rough lemon rootstock (12), generally do not have increased yields in response to supplemental irrigation under Florida conditions (A.J. Rose, personal communication, 1982).

Effects of Irrigation and GA_3 and 2,4-D on Drop and External Fruit Quality

No differences were observed in irrigated compared with unirrigated trees in fruit drop, FRF, RPF, or color, although SMC differed significantly throughout both seasons. Spray applications of GA_3 and 2,4-D, however, significantly decreased late-season and total fruit drop

Fig. 2-1. SMC and ψ_L of irrigated (■▪▪▪▪▪▪■) and unirrigated (●▬▬▬●) 'Marsh' grapefruit averaged over both sprayed and unsprayed treatments during 1980-81 and 1981-82. Each symbol is the mean of 20 measurements. The symbol ** within a date indicates a significant difference at the P = <0.01 level.

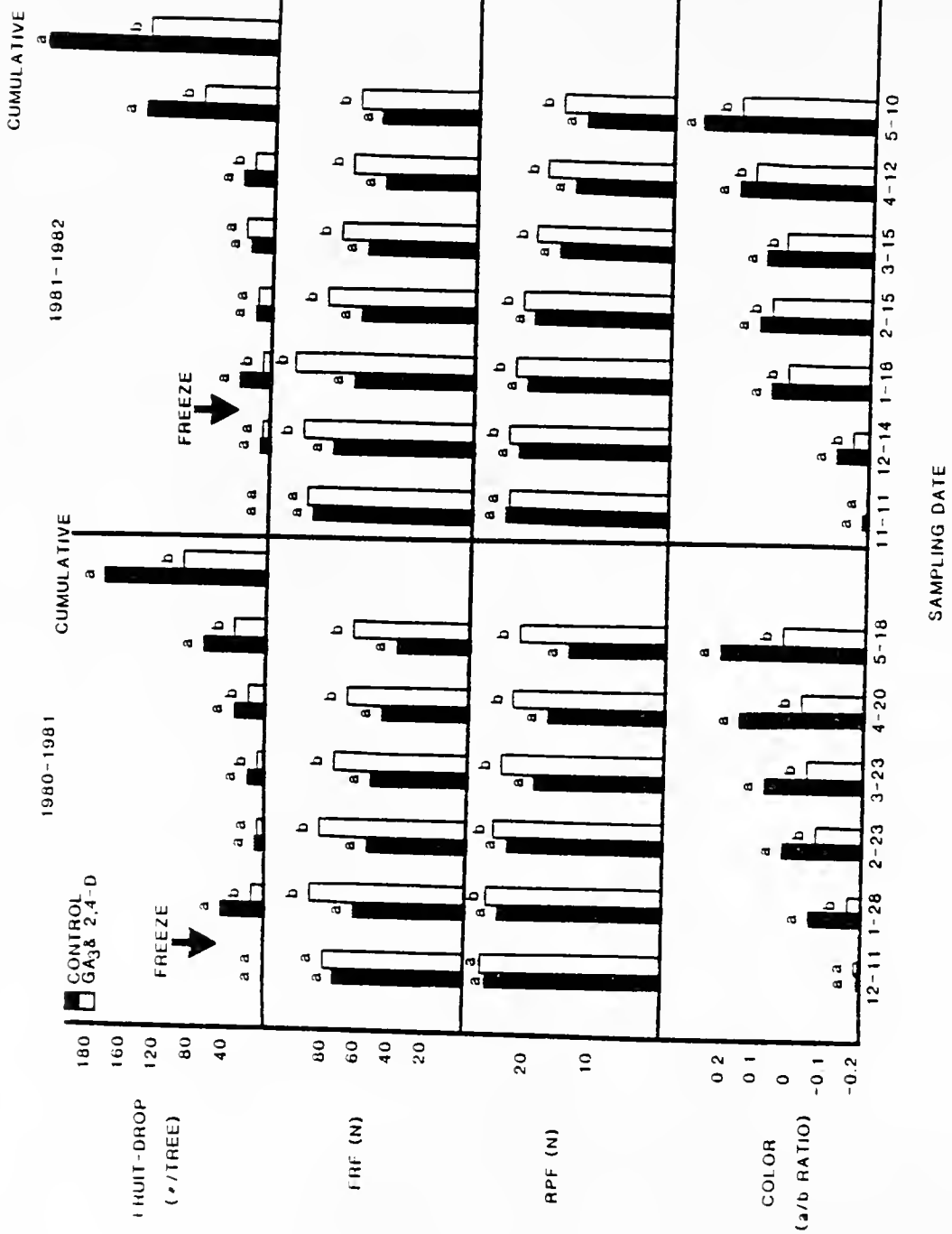

1980-1981
1981-1982
CONTROL
UNIRRIGATED
4 AM
11 AM
SMC (%)
ψ (MPa)
SAMPLING DATE
12-10
1-21
2-23
3-23
4-28
5-15
11-7
12-7
1-9
2-6
3-6
4-3
5-10

and delayed loss of FRF and RPF and development of overmature peel color both seasons (Fig. 2-2). Gilfillan et al. (35) observed that inadequate irrigation resulting in wilting decreased the efficacy of GA_3 and 2,4-D in preventing fruit drop and peel puffiness. However, they did not measure SMC or Ψ_L. Citrus trees growing in arid climates have shallower, less extensive root systems than trees in moderately high-rainfall areas with deep sandy soils (20). These data indicate that GA_3 and 2,4-D applications in areas with the latter characteristics should produce favorable responses, regardless of irrigation practices. These findings are consistent with previous reports that GA_3 and 2,4-D extend the grapefruit harvest season in Florida (2), South Africa (35), and Australia (25).

Effects of Decreased Irrigation and GA_3 and 2,4-D on Internal Fruit Quality

The TSS, percentage of acid, TSS/acid ratio, percentage of juice, weight per fruit, and incidence of seed sprouting were unaffected by GA_3 and 2,4-D application or irrigation treatments. Previous workers have reported only slight, inconsistent effects of GA_3 and 2,4-D on internal quality of grapefruit (2,25,35,59). Ali Dinar et al. (2) reported a significant decrease in seed sprouting during one season in Florida, a result not corroborated here or by Albrigo et al. (1). Ali Dinar et al. (2) did not clearly demonstrate whether it was GA_3 or 2,4-D or the combination that decreased sprouting, as all three decreased sprouting somewhat.

Fig. 2-2. Effect of GA_3 and 2,4-D on fruit drop, FRF, RPF, and peel color averaged over both irrigated and unirrigated treatments for 1980-81 and 1981-82. Each bar is the mean of 20 measurements. Different letter superscripts indicate significant differences at the $P = <0.01$ level.


1980-1981
1981-1982
CUMULATIVE
CUMULATIVE
CONTROL
GA3 & 2,4-D
FREEZE
FREEZE
FRUIT-DROP (#/TREE)
FRF (N)
RPF (N)
COLOR (a/b RATIO)
SAMPLING DATE
12-11 1-28 2-23 3-23 4-20 5-18 11-11 12-14 1-18 2-15 3-15 4-12 5-10

Effects of GA_3 and 2,4-D on Postfreeze Fruit Drop

Grove air temperatures were between -2.2° and -5.5°C for 10 hours on January 12, 1981, and between -3.9° and -5.5°C for 6 hours on January 13, 1981. Temperatures remained between -2.2° and -5.5°C for 10 hours on January 12, 1982. Severe fruit drop generally occurs when fruit are exposed to such temperature extremes; however, January fruit drop was significantly lower for GA_3 and 2,4-D treated trees, particularly after the more severe freeze of 1981 (Fig. 2-2). The difference in fruit-drop rates of treated and untreated trees was again insignificant in February, suggesting that the difference in both Januarys was a result of the freezes.

Conclusions

These experiments support previous reports of the efficacy of GA_3 and 2,4-D in extending the grapefruit harvest season by improving peel color and firmness and decreasing late-season fruit drop. Furthermore, under Central Florida conditions, presence or absence of low-volume irrigation did not alter the efficacy of GA_3 and 2,4-D in extending the harvest season of large, mature grapefruit trees. Applications of GA_3 and 2,4-D reduced postfreeze fruit drop.

CHAPTER III

PREHARVEST AND POSTHARVEST GIBBERELLIC ACID AND 2,4-DICHLOROPHENOXYACETIC ACID APPLICATIONS FOR INCREASING STORAGE LIFE OF GRAPEFRUIT

Introduction

White 'Marsh' seedless grapefruit (Citrus paradisi Macf.) has a long, September to May harvest season, is a nonclimacteric fruit with a low respiration rate and minimal starch reserves, and is unresponsive to controlled atmosphere storage (42). For nearby markets the fruit can be stored on the tree. Distant markets such as Japan, however, require postharvest storage during transport. Therefore, any preharvest or postharvest treatment that extends postharvest life is desirable.

Currently, GA_3 and 2,4-D treatments, applied preharvest or postharvest, show promise for extending grapefruit postharvest storage life. Preharvest and postharvest GA_3 treatments have the same effect; both delay overripe color development and loss of peel firmness. Preharvest 2,4-D also delays color development and loss of firmness somewhat, but primarily delays preharvest abscission. Postharvest 2,4-D indirectly decreases growth of some fungal pathogens by rendering the host grapefruit less vulnerable to pathogen entrance, particularly around and under the calyx where Alternaria citri spores exist in abundance (34,90). This is important because citrus fruits almost invariably succumb to fungal invasion before physiological breakdown renders them unmarketable (42).

Response of grapefruit to any preharvest or postharvest treatment for extending postharvest storage life could vary depending upon when during the season and time of day fruit is harvested (103), rootstock (88), tree condition, cultural and harvesting practices, and intentional or unintentional postharvest treatment (42).

Literature Review

Effects of Preharvest GA_3 and 2,4-D Sprays on External Quality of Postharvest Stored Grapefruit

Much work has been done on the effects of preharvest GA_3 and 2,4-D sprays on the preharvest quality (2,25,35,59), but there are few reports on the effects of these materials on postharvest quality. Preharvest GA_3 and 2,4-D sprays, applied at a rate of 10-20 ppm for GA_3 and 20-40 ppm for 2,4-D, have been reported to delay postharvest overripe color development and loss of firmness in California (15), Florida (1), and Australia (34,36). Similar results have been obtained with 'Shamouti' oranges in Israel (70). In contrast, Fucik (33) in Texas did not observe preharvest GA_3 and 2,4-D sprays to maintain peel firmness of 'Ruby Red' grapefruit in storage, even though treated fruit had firmer peels than controls at harvest. However, he applied GA_3 at only 1 ppm, whereas it was applied at 10-20 ppm in most other studies (1,15,33,34,36).

Grapefruit invariably succumb to fungal invasion before physiological breakdown renders them unmarketable (42). Therefore, postharvest storage life generally depends on an interaction between physiological and pathological factors. Preharvest sprays of GA_3 and 2,4-D (33) and 2,4-D alone maintain the calyx in a more juvenile state, thus hindering the entrance of Alternaria citri spores, which cause stem-end rot.

Bevington (10) in Australia reported less green mold (Penicillium digitatum) wastage of navels in cold storage when they had been treated preharvest with GA_3.

Effects of Preharvest GA_3 and 2,4-D on Internal Quality of Postharvest Stored Grapefruit

Except for slight increases in percentage of juice, the internal quality of 'Marsh' grapefruit does not change greatly with extended storage (86). Gallasch (34) reported a decrease in percentage of acid of stored grapefruit that had been treated preharvest with GA_3 alone. Monselise and Sasson (70) contradicted this with a report of increased percentage of acid in stored 'Shamouti' oranges that had been treated preharvest with GA_3 and 2,4-D. They attributed this increase to 2,4-D, as GA_3 alone did not produce this result.

There are no reports of preharvest GA_3 sprays and 2,4-D sprays reducing the incidence of seed sprouting and granulation in grapefruit stored postharvest.

Effects of Postharvest GA_3 and 2,4-D Treatments on External Quality of Postharvest Stored Grapefruit

There are no reports on the use of postharvest GA_3 or GA_3 and 2,4-D treatments for grapefruit. Postharvest applications of GA_3 maintained 'Shamouti' orange (37) and lemon (69) peel in a more juvenile state during storage. El-Nabawy et al. (24) reported that postharvest GA_3 dips retarded color development, decreased weight loss, and decreased the percentage of discards in stored 'Valencia' oranges. Postharvest 2,4-D treatments decreased weight loss and percentage of discards in stored 'Valencia' oranges (24), maintained the calyx in a

juvenile state in 'Marsh' grapefruit (90), 'Kinnow' mandarin, and 'Eureka' lemons (29), and decreased losses due to Alternaria in 'Marsh' grapefruit (89,90). Application rates were higher than those of preharvest sprays, ranging from 100 to 2000 ppm for GA_3 and from 500 to 2000 ppm for 2,4-D (15,24,29,89,90).

Effects of Postharvest GA_3 and 2,4-D Treatments on Internal Quality of Postharvest Stored Grapefruit

Internal quality of 'Marsh' grapefruit is not altered greatly in extended cold storage (86) except for a slight increase in the percentage of juice. Postharvest GA_3 and 2,4-D treatments do not produce any consistent, significant changes (15,68). El-Nabawy et al. (24) reported slight increases in TSS and decreases in percentage of acid of stored 'Valencia' oranges that were treated postharvest with 2,4-D or GA_3.

There are no reports of postharvest GA_3 and 2,4-D treatments, alone or combined, reducing seed sprouting or granulation in grapefruit stored postharvest.

The objective of these experiments was to compare the ability of preharvest and postharvest GA_3 and 2,4-D treatments to maintain grapefruit quality in storage.

Materials and Methods

Two sets of 40 'Marsh' white seedless grapefruit (Citrus paradisi Macf.) trees on rough lemon (C. jambhiri Lush.) rootstock from the same grove were used successively during the 1980-81 and 1981-82 seasons. Trees were growing in Astatula fine sand, were hedged north-south in 4.5×9.1-m spacing, and had low-volume undertree

irrigation. Normal grove production practices were followed. The 40 trees were divided into a 20-tree preharvest control treatment and a 20-tree preharvest spray treatment. Completely randomized single-tree plots were used during 1980-81 and two-tree plots during 1981-82. When all fruit were slightly past colorbreak on December 12, 1980, and November 11, 1981, approximately 50 liters of an aqueous combination of GA_3 (20 ppm), 2,4-D (20 ppm), and X-77 surfactant (0.025 v/v) were sprayed on each preharvest treatment tree. Control trees were not sprayed.

On January 26, March 23, and May 18, 1981, and January 18, March 15, and May 10, 1982, approximately 60 fruit per tree were randomly harvested from the four quadrants of each tree. Blemished fruit were removed, and the remainder was washed on a packinghouse line and air dried. Initial seed sprouting counts were done on 20 fruit per tree. An additional 20 fruit per tree were used to obtain initial peel color measurement by the Hunter Color Difference Meter (47), peel puncture strength by the Instron penetrometer (48), and juice quality by automated systems analysis (23). Peel color was tested on one location per fruit and peel firmness on four. The remaining fruit were combined within each of the two preharvest treatments, control and preharvest spray, and then divided into two equal lots within these treatments. One lot from each field treatment was dipped for 1 minute in an aqueous combination of GA_3 (100 ppm), 2,4-D (500 ppm), and X-77 surfactant (0.025% v/v). These preharvest and postharvest treatments produced four storage treatments: a control treatment without preharvest spray or postharvest dip, a treatment with postharvest dip only, a treatment with preharvest spray only, and a

treatment with both spray and dip. All fruit received 1000 ppm thiabendazole (TBZ), were dried and waxed on a packinghouse line, were packed into 20-kg cartons (four replications per storage treatment), and were stored at 15.5°C and 95% relative humidity (67) for 12 weeks. Color readings on 20 randomly selected fruit per carton and decay checks on all fruit were done weekly. At 12 weeks, peel puncture resistance testing and juice analysis were repeated on two 20-fruit samples per treatment and seed sprouting was counted on 20 fruit per treatment.

The Hunter Color Difference Meter measures chromaticity dimensions of colors and expresses them as "a/b" ratios; "a" measures redness when positive and greenness when negative, and "b" measures yellowness when positive and blueness when negative. The ratio indicates proportions of the above colors (47). A pale yellow, marketable grapefruit has an "a/b" ratio ranging from -0.1 to 0.2.

The Instron penetrometer determines peel puncture resistance in newtons (N) required to puncture a 0.63 cm hole in the peel using a flat head moving at 20 cm per min. N is a unit of force independent of mass and is not comparable to the mass unit, kg.

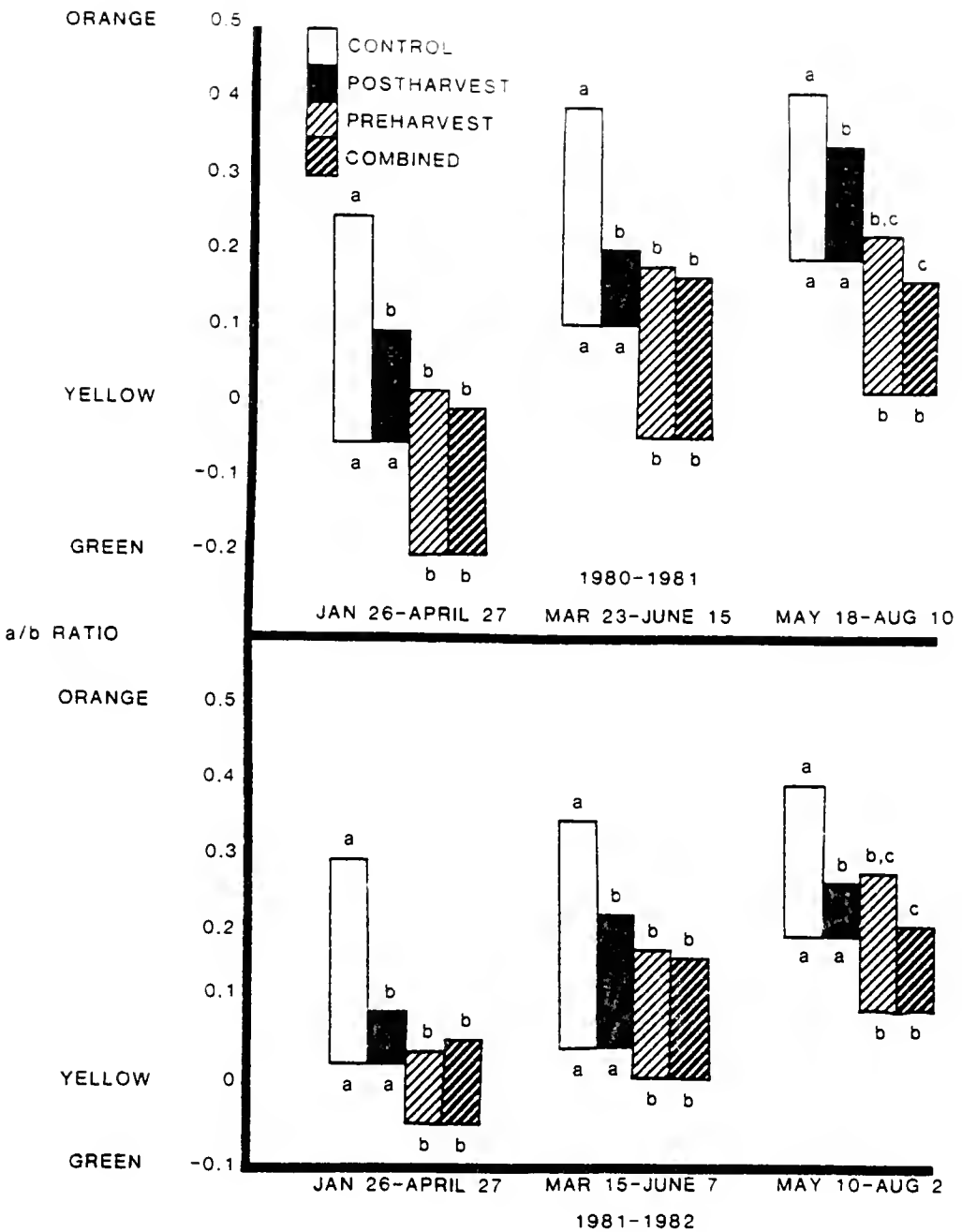
Data were analyzed using analysis of variance and Duncan's multiple range test.

Results and Discussion

Effects on Color

The GA_3 and 2,4-D delayed overripe peel color development when applied before or after harvest (Fig. 3-1). Fruit from trees treated preharvest had significantly lower "a/b" ratios than controls on all six

Fig. 3-1. Comparison of preharvest, postharvest, and combined preharvest and postharvest GA_3 and 2,4-D treatments on peel color at harvest (bottom of bar) and after 12 weeks of storage (top of bar). Treatment means within a harvest date at bottom (harvest) and top (after storage) separated by Duncan's multiple range test, $P = <0.05$. Means are an average of 80 values.

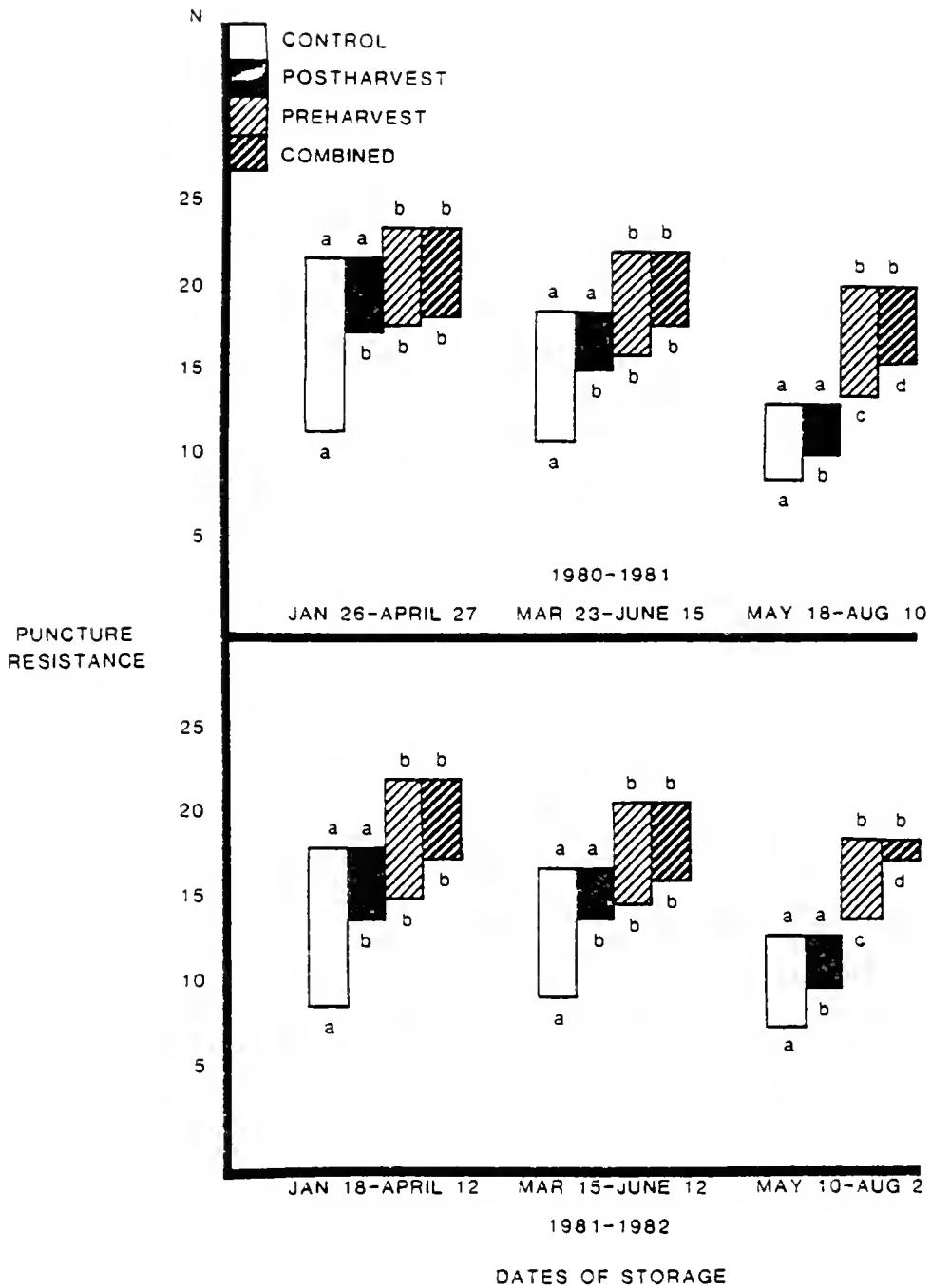

ORANGE
YELLOW
GREEN
a/b RATIO
0.5
0.4
0.3
0.2
0.1
0
-0.1
-0.2
CONTROL
POSTHARVEST
PREHARVEST
COMBINED
JAN 26-APRIL 27
MAR 23-JUNE 15
MAY 18-AUG 10
1980-1981
JAN 26-APRIL 27
MAR 15-JUNE 7
MAY 10-AUG 2
1981-1982

harvest dates, confirming an earlier Florida report (2). Fruit that had received any of the GA_3 and 2,4-D treatments still had significantly lower "a/b" ratios than controls after 12 weeks in storage. All treatments were equally effective from January to March in delaying overripe peel color, but in May, combined preharvest and postharvest treatment was significantly more effective than postharvest, but not preharvest, treatment. Therefore, prior to April, there was no advantage to combined preharvest and postharvest treatment, as both delayed color development equally well alone. Preharvest or combination preharvest and postharvest treatment was best for fruit harvested in May, as either produced significantly less overripe color development. If only a single GA_3 and 2,4-D application is possible, preharvest is preferable, as it consistently produced a lower "a/b" ratio than postharvest applications (2,25,35,59).

Effects on Peel Puncture Resistance

The GA_3 and 2,4-D maintained peel puncture resistance when applied either preharvest or postharvest (Fig. 3-2). Fruit from trees that were treated preharvest had significantly higher puncture resistance than controls, confirming earlier data reported by Ali Dinar et al. (2). However, for harvested fruit they reported puncture resistance from 6.14 to 8.90 N compared with the 13.75 to 21.50 N here. Our values agree with two other Florida reports (F.S. Davies and M.A. Ismail, 1979, and A. J. Rose, 1979, both personal communications) and one Australian report (25). Fruit treated before or after harvest, or at both times, had consistently higher puncture resistance after 12 weeks in storage than the controls did. All treatments were equally

Fig. 3-2. Comparison of preharvest, postharvest, and combined preharvest and postharvest GA_3 and 2,4-D treatments on peel puncture resistance (higher values = higher resistance) at harvest (top of bars) and after 12 weeks of storage (bottom of bars). Treatment means within a harvest date at top (at harvest) and at bottom (after storage) separated by Duncan's multiple range test, $P = <0.05$. Means are an average of 80 values.

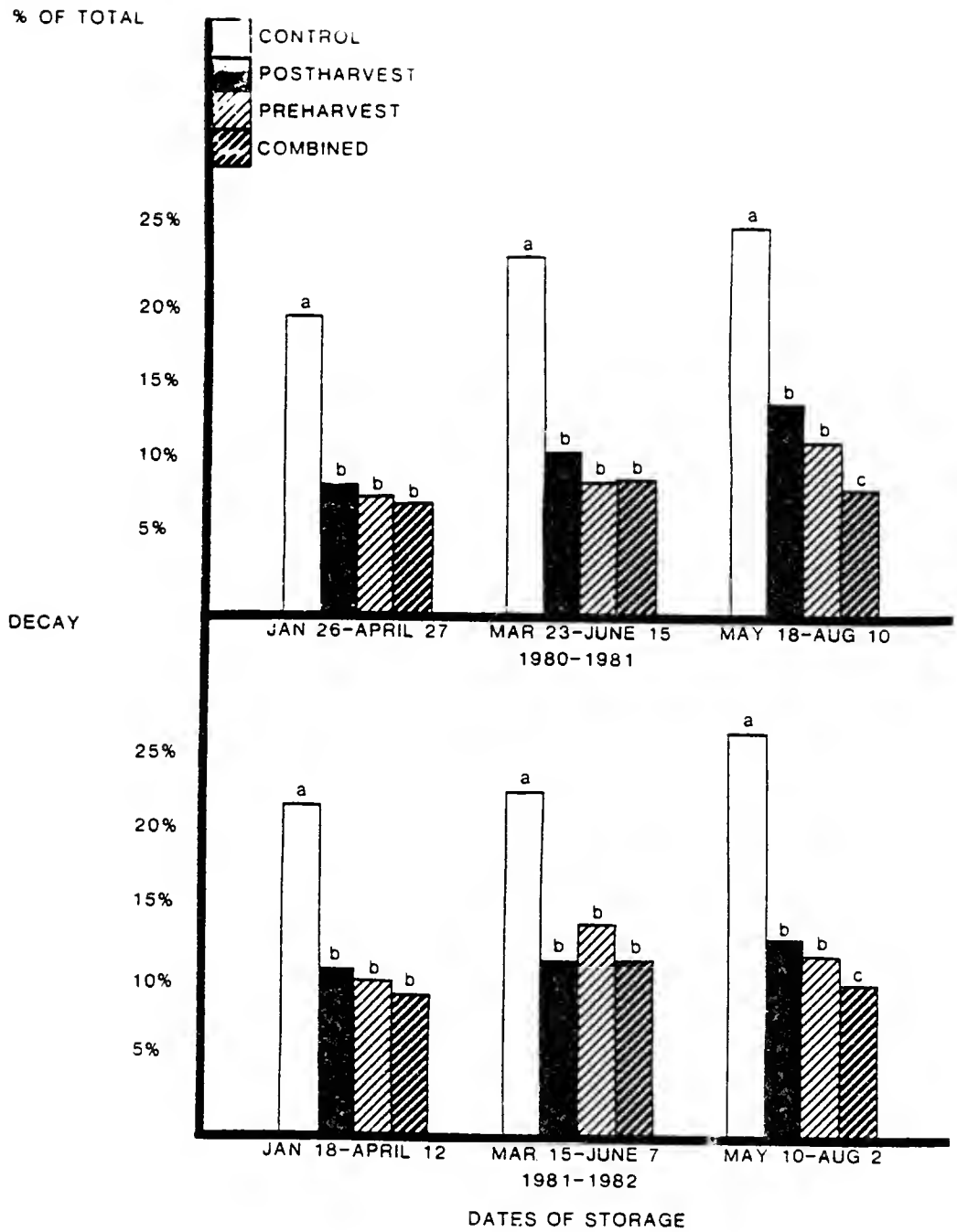

N
CONTROL
POSTHARVEST
PREHARVEST
COMBINED
25
20
15
10
5
PUNCTURE RESISTANCE
1980-1981
JAN 26-APRIL 27
MAR 23-JUNE 15
MAY 18-AUG 10
1981-1982
JAN 18-APRIL 12
MAR 15-JUNE 12
MAY 10-AUG 2
DATES OF STORAGE

effective for January and March harvests, but for May harvests, combined preharvest and postharvest treatment was significantly better than postharvest treatment. Therefore, through March, there was no advantage to combined preharvest and postharvest treatment, as a single preharvest or postharvest treatment maintained peel puncture resistance as well. After March, preharvest or combined preharvest and postharvest treatment was more effective than a postharvest treatment. However, if only a single GA_3 or 2,4-D treatment is possible, preharvest treatment is preferable, as it also decreases late-season fruit drop losses (2,25, 32,35).

Effects on Decay

The GA_3 and 2,4-D treatments decreased decay in storage (Fig. 3-3). There were no significant differences between preharvest, postharvest, and combined preharvest and postharvest applications; all resulted in significantly less decay than in untreated control fruit. Onset of decay was 1 to 2 weeks earlier and stem-end rot was more frequent in control fruit. These data agree with earlier reports of 2,4-D's ability to decrease grapefruit decay, particularly stem-end rot, when applied postharvest (89,90,98). Preharvest or postharvest applications of GA_3 have been reported to delay peel senescence, thereby maintaining peel quality in storage (69). The results here suggest that these decreases in decay are partially a result of better peel condition, rendering the fruit less susceptible to injury and therefore to invasion by fungal pathogens. This would explain why preharvest and postharvest treatments function equally well.

Fig. 3-3. Comparison of preharvest, postharvest, and combined preharvest and postharvest GA_3 and 2,4-D treatments on decay after 12 weeks in storage. Treatment means within a storage period separated by Duncan's multiple range test, $P = <0.05$. Means are an average of 80 values.


% OF TOTAL
CONTROL
POSTHARVEST
PREHARVEST
COMBINED
25%
20%
15%
10%
5%
DECAY
a
b
b
b
a
b
b
b
a
b
b
c
JAN 26-APRIL 27
MAR 23-JUNE 15
MAY 18-AUG 10
1980-1981
a
b
b
b
a
b
b
b
a
b
b
c
JAN 18-APRIL 12
MAR 15-JUNE 7
MAY 10-AUG 2
1981-1982
DATES OF STORAGE

Effects on Internal Quality

Juice content, total soluble solids (TSS), percentage of acid, TSS/of acid ratio, and individual fruit weights did not display any significant, consistent differences due to treatment either at harvest or after 12 weeks in storage (data not shown). This is consistent with earlier reports for preharvest (2,25,35,59) and postharvest (15,24,68) treatments. Seed sprouting was unaffected by all treatments during either season. This disagrees with an earlier Florida report (2) and agrees with another (1).

Conclusions

These results confirm earlier reports that preharvest GA_3 and 2,4-D sprays maintain grapefruit peel quality on the tree (2,25,35) and in storage (1) without affecting internal quality (2,25,35). Preharvest GA_3 and 2,4-D application was the best treatment under most circumstances. Overripe color development and loss of puncture resistance were retarded more effectively through storage when combined GA_3 and 2,4-D was applied preharvest. This is consistent with Goldschmidt and Eilati's report of GA_3 delaying color development more effectively in unharvested than harvested 'Shamouti' oranges (37). Preharvest GA_3 and 2,4-D produced better peel quality in tree-stored fruit and delayed abscission (2,25,32,35), thereby extending the harvest season. Postharvest GA_3 and 2,4-D dips, although less expensive than preharvest sprays and equally effective in maintaining peel quality and decreasing decay of stored fruit harvested through March, will not enable extension of the harvest season. Applying both a preharvest spray and a postharvest dip was advantageous only for fruit harvested in May. Otherwise, a preharvest spray or a postharvest dip had equal effects.

Despite GA_3's effectiveness in slowing peel senescence in grapefruit, seed germination and granulation in late season fruit still remain a problem. Both result in development of off flavors and lower grade of fresh market and processing fruit.

CHAPTER IV

UPTAKE, TRANSLOCATION, PERSISTENCE, AND METABOLISM OF GIBBERELLIC ACID IN GRAPEFRUIT

Literature Review

Uptake, Translocation, and Persistence of ^{14}C-GA_3

Little is known about uptake and transport of gibberellins in grapefruit. Gibberellin uptake and transport studies using excised sections of coleoptiles, stems, or petioles indicate that gibberellins are readily absorbed, their movement is passive and nonpolar in xylem and phloem at rates of 5-25 mm per 12-hour period, and interchange occurs between xylem and phloem (7,11,13,66,72,113). These results suggest that gibberellins are transported with carbohydrates. Acropetal and basipetal polar movement of gibberellins occurs in coleus petioles (52), stems (53), and subapical root sections of several higher plants (3,7, 53,72,78). However, these may not be examples of true polar movement, but may simply be movement toward a growth center (78,87,108).

Goldschmidt and Eilati (37) applied GA_3 to the flavedo of 'Shamouti' oranges before and after harvest. Delay of peel color development indicated that GA_3 was absorbed by both attached and detached fruits, although this effect was more marked and persistent in the former. Delay of peel color development beyond the area of flavedo application suggested lateral diffusive movement of the GA_3. This movement was further and more toward the stylar end in attached than detached fruit. Goldschmidt and Galili (38) applied ^{3}H-GA_3 to detached

'Shamouti' orange peels and recovered measurable amounts of radioactivity from the flavedo after 5 days of air or ethylene storage. In a later study (39) they applied ^{14}C-GA_3 to 'Valencia' oranges on the tree and recovered only 2% of applied radiocactivity from the flavedo after 24 hours.

Evidence for localization of GA_3 following uptake and translocation within citrus peel is indirect. Exogenously applied GA_3 delays loss of chlorophyll, RNA, and proteins (30,31) in the flavedo and maintains a more compact structure in the albedo (69), suggesting that it or its metabolites are localized in these areas. Some gibberellin biosynthesis and metabolism occurs in chloroplasts and leucoplasts (72). Ohlrogge et al. (76) and Silk and Jones (94) suggest that gibberellins are compartmentalized in vacuoles.

Evidence for persistence of exogenously applied GA_3 in citrus peel is both indirect and direct. Sprays of GA_3 delay peel color development and loss of rind firmness for as long as 7 months after application. This may be an indirect indication of GA_3's persistence in citrus peel (16). Direct evidence of persistence has been demonstrated by Jordan et al. (57), who found 0.10-0.15 ppm GA_3 in lemon peel 7 days after application. Goldschmidt and Galili (39) applied ^{14}C-GA_3 to 'Valencia' oranges on the tree and recovered 1% of the applied radioactivity after 100 days. However, until the presence of endogenous GA_3 is established in citrus peel, physiochemical methods of detecting GA_3 cannot conclusively demonstrate the persistence of absorbed GA_3.

Metabolism of ^{14}C-GA_3

Little is known about gibberellin metabolism in higher plants. Glycosylation, the attachment of a glucose molecule through an ether bond forming a water-soluble gibberellin glucoside, is common with gibberellins in higher plants. Water-soluble, "bound," gibberellin-like substances were discovered in higher plants in the 1960s (58,65,66,74). Hydrolysis with acid, ficin, emulsin, papain, or B-D-glucosidase (6,7, 9,45,54,65,74,82,110) released free gibberellins and glucose. "Bound" is now reserved for unidentified gibberellin-like substances, whereas "conjugated" describes complexes in which both gibberellin and its bound counterpart are known (62,92).

Conjugated gibberellins are much less active than free gibberellins (8,93,103,109,111). The deactivation of free gibberellins by glycosylation (7,62,72), the frequency and rapidity with which it happens to applied gibberellins (7,11,113), and its reversibility (9,75,77,92) suggest that glycosylation is a regulatory mechanism. Possible functions of deactivation by glycosylation could be to aid transport and storage of free gibberellins (62).

Primary evidence for glycosylation as an aid to gibberellin transport comes from xylem and phloem feeding studies. Water-soluble glucosides can be transported in xylem and phloem more readily than less soluble free gibberellins. Bowen and Wareing (11) reported that a portion of ^{14}C-GA_3 applied to willow shoots was quickly converted to conjugates once in xylem and phloem. The major fraction of gibberellins in spring bleeding sap of deciduous trees was in the form of gibberellin conjugates (92).

Primary evidence for glycosylation of gibberellins as a storage form of free gibberellins comes from studies with seeds. Immature seeds were injected with radiolabeled gibberellins, and relative levels of radioactive gibberellin conjugates and free gibberellins were observed. Barendse et al. (9) and Sembdner et al. (92) reported partial conversions of free gibberellins into conjugates prior to and through maturity, then a conversion of these conjugates into free gibberellins during germination. Sembdner et al. (92) also observed a conversion of applied GA_3 to its conjugated form in developing bean pods. These studies are consistent with reports by Hashimoto and Rappaport (44) and Pegg (77), who determined levels of endogenous gibberellins in developing seeds. Endogenous free gibberellins steadily decreased to very low levels at maturity as endogenous conjugated gibberellins increased. The situation reverses with germination. Pegg (77) suggested that this reversal is caused by hydrolysis of conjugated gibberellins. Barendse (6) observed enhanced conversion of ^{3}H-GA_3 to a water-soluble compound when ^{14}C-glucose was applied simultaneously to Japanese morning glory plants, suggesting that GA_3-glucoside formation was enhanced in the developing seed pods when there was available glucose to conjugate with.

Interconversions of free and conjugated gibberellins also occur in peaches and apricots (50,51,72). Free gibberellins are most abundant in tissue during periods of maximum growth (50,51), then decrease sharply relative to conjugated forms as growth decreases (51,72). These data suggest that each tissue maintains its own supply of gibberellins, although other studies suggest that bound gibberellins in mature seeds are available to other fruit tissues (49,106).

Endogenous GA_3-glucosides have been found in several higher plants (66,72,92,104,111,112). Moreover, exogenous application of ^{14}C-GA_3 forms a glucoside indistinguishable from the endogenous one (7,22,92,93). Glucosides of gibberellins are difficult to separate from acidified aqueous solutions with ethyl acetate; however, glucosides can be partitioned from aqueous solutions with N-butanol (7,72,101). Goldschmidt and Galili (38,39) applied radiolabeled GA_3 to 'Shamouti' and 'Valencia' orange peels. In both experiments, diethyl ether or ethyl acetate partitions contained approximately one half of the recovered radioactivity on all sampling dates. The other half remained in the aqueous fraction. The organic fractions co-chromatographed with a GA_3 standard on silica gel H-coated plates. Thin-layer chromatography peaks decreased in height and broadened with each successive sampling date (1, 10, 100 days), suggesting catabolism of the ^{14}C-GA_3. Total recovered radioactivity decreased, but the ratio of ethyl acetate and water-soluble fractions remained relatively constant, indicating that ^{14}C-GA_3 glucosides recovered in the water-soluble fraction may be slowly converted to ^{14}C-GA_3 soluble in the ethyl acetate fraction. Silk and Jones (94) proposed a similar situation with ^{3}H-GA_1 in excised lettuce hypocotyls. Alternative explanations might be that the ethyl acetate-soluble fraction consists of free ^{14}C-GA_3 (14,22) or ^{14}C-GA_3 sequestered in an organelle (76), and as it is used or released it is degraded into a water-soluble form. However, without N-butanol partitioning to separate glucosides, or positive identification via gas chromatography-mass spectroscopy, these possibilities cannot be verified.

Chapters II and III of this dissertation demonstrated the ability of exogenously applied GA_3 to maintain preharvest and postharvest peel

quality of 'Marsh' grapefruit. However, little is known about the fate of exogenously applied ^{14}C-GA_3. Therefore, this study will determine the uptake, translocation, and beginning metabolism of ^{14}C-GA_3 applied to attached 'Marsh' grapefruit peel and leaves.

Materials and Methods

Application of ^{14}C-GA_3 to Attached Fruit and Leaves

Unlabeled GA_3 was obtained from Abbott Laboratories, Chicago, Illinois (Pro-Gibb®, 3.91% in isopropyl alcohol), and ^{14}C-GA_3 (1,7,12, 18) (^{14}C-GA_3, 14 mCi/mMol) was purchased from The Radiochemical Centre, Amersham, England. The original ethyl acetate solution of ^{14}C-GA_3 was reduced to dryness under vacuum at 37°C and dissolved in 25 ml of an aqueous solution of 1.0% isopropanol, 0.025% X-77, and 0.01 ml of Pro-Gibb®, which produced a stock solution of 20 ppm GA_3 solution containing 1.65×10^5 disintegrations per minute (DPM) per 200-µl aliquot.

Sixty-six mature grapefruit with three or more subtending leaves not more than 10 cm from the fruit were randomly selected, no more than two per tree, from 40 container-grown 3-year-old 'Marsh' grapefruit (Citrus paradisi Macf.) on Milam (C. jambhiri hybrid ?) rootstock. Thirty-three fruit from different trees had ^{14}C-GA_3 applied to the fruit surface and 33 fruit from different trees had ^{14}C-GA_3 applied to both surfaces of the subtending leaves. Each fruit was visually divided into four quadrants, and 50 µl of the stock solution were evenly dotted on the surface with a microsyringe and spread about with the tip. Leaf application was done in the same manner. Each fruit and leaf set

received a total of 1.65×10^5 DPM. Application was done between 10 and 11 AM in full sunlight at temperatures of 28-32°C and relatively humidities of 59-84%.

Application of ^{14}C-GA_3 to Detached Fruit

Mature grapefruit were randomly harvested from 40 container-grown 3-year-old 'Marsh' white seedless grapefruit trees on Milam rootstock. Fruit were washed with tepid water, air dried, and divided into three replicate sets; ^{14}C-GA_3 was then applied as previously described. Fruit were held at 26°C and 65-89% relative humidity in fluorescent light until sampled at 0, 1, 2, 8, 24, and 48 hours. Sampling was done as described below for attached fruit.

Extraction of Radioactivity

Three replicates of treated fruit with untreated leaves and three replicates of untreated fruit with treated leaves were harvested 0, 1, 2, 4, and 8 hours, 1 and 4 days, and 1, 2, 4, and 8 weeks after ^{14}C-GA_3 application. Surface ^{14}C-GA_3 was removed by washing three times with 95% ethanol. A 5-ml aliquot of the combined washes was added to 15 ml of Aquasol II (New England Nuclear) liquid scintillation cocktail (LSC) for quantification of unabsorbed ^{14}C-GA_3. Woody tissue between fruit abscission zone and proximal end of the petiole was kept separate from woody tissue proximal to the leaf cluster. The flavedo was removed with a potato peeler, albedo with a sharp knife, and seeds with tweezers. Juice was extracted with a Wearever mechanical juicer, homogenized in a Lourdes grinder for 2 minutes, and filtered with Whatman #1 paper, and a 5-ml aliquot was combined with 15 ml of LSC. Twigs were

homogenized three times with 25 ml of 95% ethanol and filtered with suction through Whatman #1 paper. The three twig filtrates were combined and partitioned against 150-ml volumes of petroleum ether until clear, reduced to 5 ml under vacuum at 37°C, and combined with 15 ml LSC. Radioactivity was recovered from albedo, flavedo, and leaves, using a modification of the procedure developed by Wheaton and Bausher (107) (Fig. 4-1). A polyvinylpyrrolidone (PVP) column was added after the ion exchange column to remove phenolics that might interfere with later bioassays (73). All samples were counted in a Beckman LS 5800 Series tabletop counter three times for 10 minutes and the values averaged. Efficiency was approximately 90%, quench negligible and disregarded, and a 38 counts per minute (CPM) background subtracted. All recoveries were corrected to percentage of DPM applied.

High-Performance Liquid Chromatography of Leaf, Albedo, and Flavedo Extracts

Separation of metabolites in leaf, flavedo, and albedo extracts was done with a Waters Associates modular high-performance liquid chromatography (HPLC) system (107). Column was a Waters u-Bondapak phenyl (300×3.9 mm) reversed-phase analytical column held at 30°C with C18 precolumn. Injector was U6K connected to twin Waters pumps, all controlled by a Model 660 solvent programmer. Solvent for pump A was 0.2% ammonium acetate (NH_4Ac) at pH 5.6, made with 2 ml of acetic acid (HAc) in 1000 ml of deionized H_2O. The pH was adjusted with dilute ammonium hydroxide (NH_4OH), and the final solution was filtered with suction and agitation through a 0.45 μm Gelman Acopor membrane filter. The solvent for pump B was 50% pump A solvent and 50%, 95% ethanol

EXTRACTION OF ALBEDO, FLAVEDO, AND LEAVES

Efficiency (% Recovered)		
100%	Entire Flavedo, Albedo, or Leaves	
	1. Drop in boiling 95% ethanol, 5 ml per 1 gm fresh weight	
	2. Cool to room temperature in an ice bath	
	3. Homogenize 2 minutes	
	4. Filter with Whatman #1 paper	
	5. Reextract solids ×3 with 100 ml of 80% ethanol	
96.7%	Combined Filtrate	Solids
	6. Partition until clear with 150 ml of petroleum ether	Discard
95.4%	Ethanol Phase	Pet. ether phase
	7. Reduce volume in vacuo to less than 10 ml at 37°C	Discard
	8. Make up to 20 ml of 50% ethanol solution	
	9. Centrifuge at 4200 ×g for 10 minutes	
	10. Resuspend pellet with 5 ml of 50% ethanol; recentrifuge	
94.4%	Combined Supernatant	

Ion-exchange preliminary separation by column chromatography

Sephadex A-25 DEAE (Ac- form)

11. Elute with 50 ml of 15% acetic acid in 50% ethanol

12. Reduce to dryness in vacuo at washing with 95% ethanol

13. Solubilize in 20 ml of methanol

93.6% Eluate

Removal of phenolic compounds by polyvinylpyrrolidone column chromatography

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Insoluble polyvinylpyrrolidine (in excess methanol)

14. Elute with 50 ml of MeOH ×3

92.7% Eluate

15. Reduce to dryness in vacuo at 37°C

16. Vacuum pump for 15 minutes

17. Dissolve in 2 ml of HPLC pump A solvent

18. Pass through Millipore AP prefilter and 0.02 μm Gelman Metricel membrane in syringe

19. Store in freezer

91.2% Final Extract

Fig. 4-1. Flow diagram of extraction procedure for ^{4}C-GA_3 in grapefruit albedo, flavedo, and leaves. Modification of procedure of Wheaton and Bausher (73,107).

filtered together as described. Pumps were programmed to deliver a linear gradient progressing from 100% solvent A to 100% solvent B over 50 minutes. The flow rate was 1.5 ml per minute. Each 2.0-ml sample injection was repeated three times. Fractions were collected every minute in vials containing 10 ml of LSC. Only fractions 1-20 were collected, as preliminary trials recovered little radioactivity in fractions 21-50. Fractions 21-50 were collected in bulk, reduced to dryness in vacuo at 37°C, dissolved in 10 ml of LSC, and read as a single sample.

B-D-glucosidase Hydrolysis of Radioactive Fractions

Fractions containing radioactivity were collected, reduced at 37°C repeatedly with 95% ethanol to remove HPLC solvent salts, and dissolved in 1 ml of 100 mM PO_4 buffer at pH 6.5. Fractions were combined with 1 ml of purified B-D-glucosidase (Sigma Ltd.) at 5.7 units per milliliter and incubated in a 37°C water bath for 3 hours. Samples were then reduced at 37°C, dissolved in 80% methanol, and centrifuged at 4200 ×g for 15 minutes to remove denatured enzyme. The supernatant was reduced to dryness in vacuo at 37°C, redissolved in 1 ml HPLC solvent A, passed through a Millipore AP prefilter and 0.2 Gelman Metricel membrane in a syringe, and rechromatographed to determine if hydrolysis altered retention time.

N-butanol Partition of Radioactive Fractions

Fractions containing radioactivity were collected, dried repeatedly at 37°C with 95% ethanol to remove residual ammonium acetate salts from the HPLC solvents, and dissolved in 5 ml of 50% methanol. This was reduced at 37°C to approximately 2 ml, adjusted to pH 2.5 with dilute

HCl, and partitioned as described by Russell (85). Partition phases were combined with 10 ml of LSC. The partitioning procedure separated free gibberellins from their ether and ester glucosides and the polar metabolites (Fig. 4-2).

Results and Discussion

Uptake, Translocation, and Persistence of Peel-Applied ^{14}C-GA_3

Uptake of peel-applied ^{14}C-GA_3 began immediately after application, was most rapid within 1 hour, and slowed between 1 and 2 hours after application (Fig. 4-3). The amount of radioactivity decreased in the peel during the next 8 weeks. Radioactivity was present in twigs within 4 hours, reaching subtending leaves between 4 and 8 hours past application. Radioactivity remained fairly constant, increasing in twigs through 4 weeks. Leaves accumulated radioactivity through 1 week with a subsequent decrease through 8 weeks. Residual ^{14}C-GA_3 on the peel surface decreased sharply within 1 hour of application, remained fairly stable through 4 days, then decreased steadily through 8 weeks. No radioactivity was recovered from seeds or fruit pulp.

Uptake of peel-applied ^{14}C-GA_3 to detached fruit was comparable to that of attached fruit (Fig. 4-4). Uptake began within 1 hour of application and continued for 8 hours. The radioactivity recovered decreased from 8 through 48 hours. No radioactivity was recovered from seeds or juice.

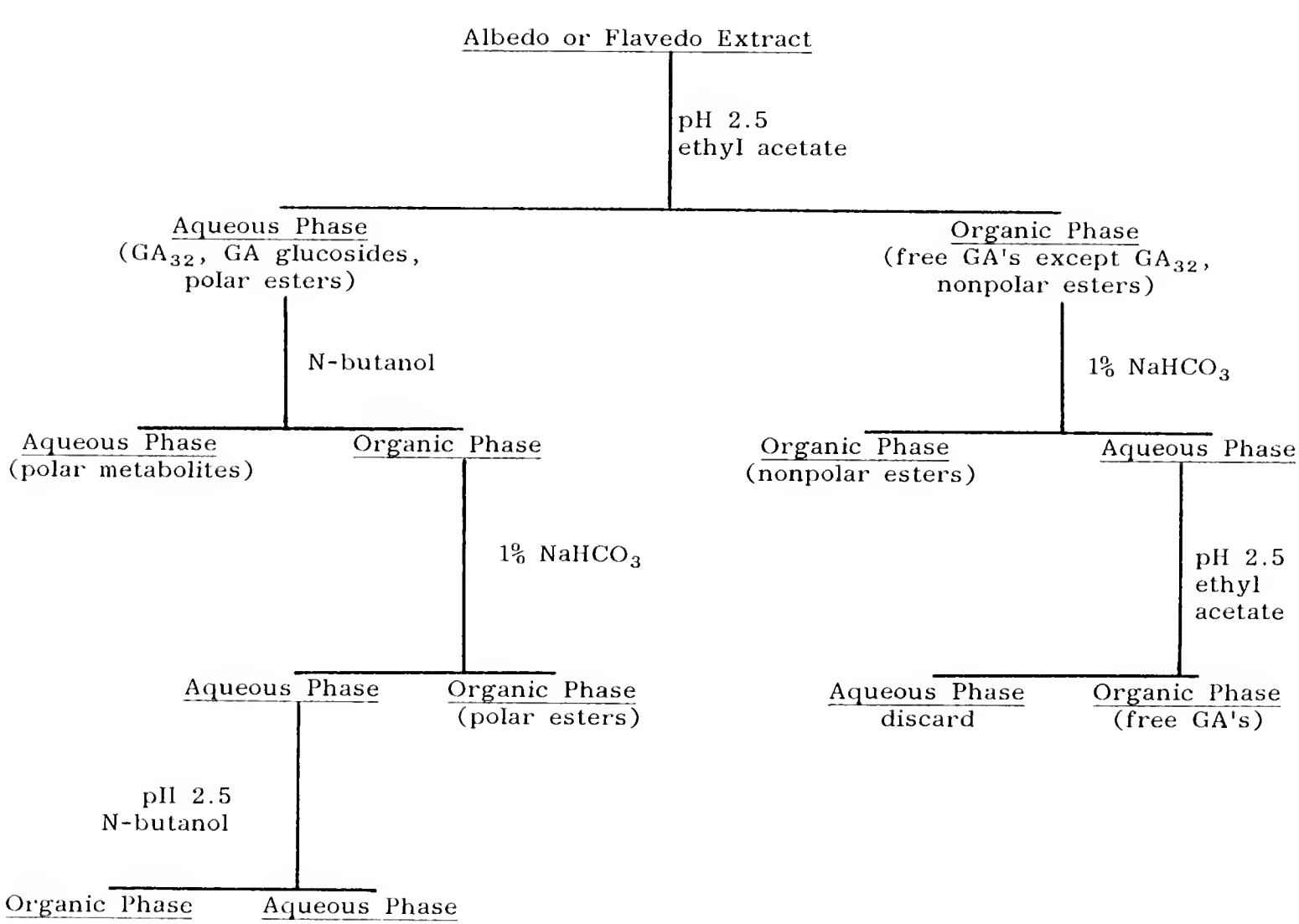


Fig. 4-2. N-butanol extraction procedure for free gibberellins, their esters and glucosides (85).

Fig. 4-3. Recovery of peel-applied radioactivity from grapefruit (albedo and flavedo combined), subtending leaves, and twigs. Means of nine replications per time.

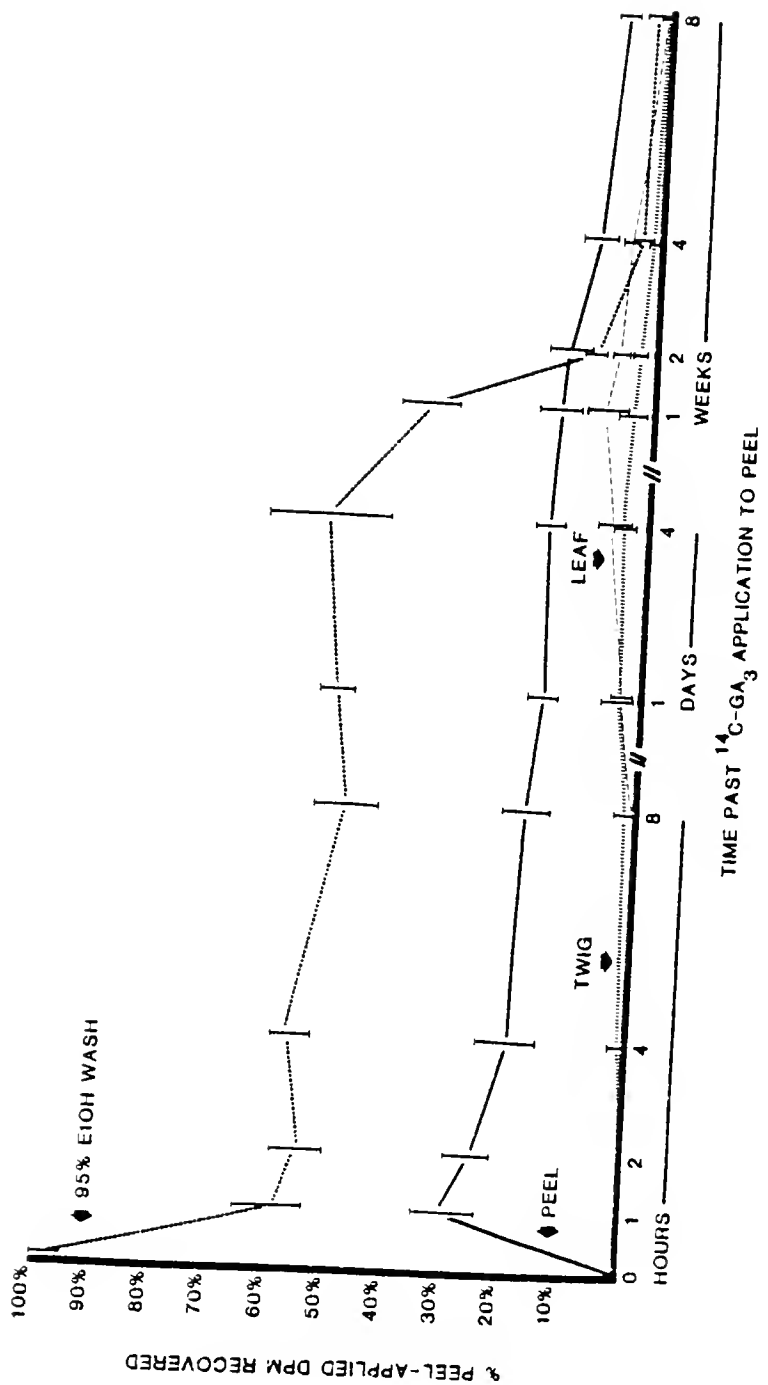
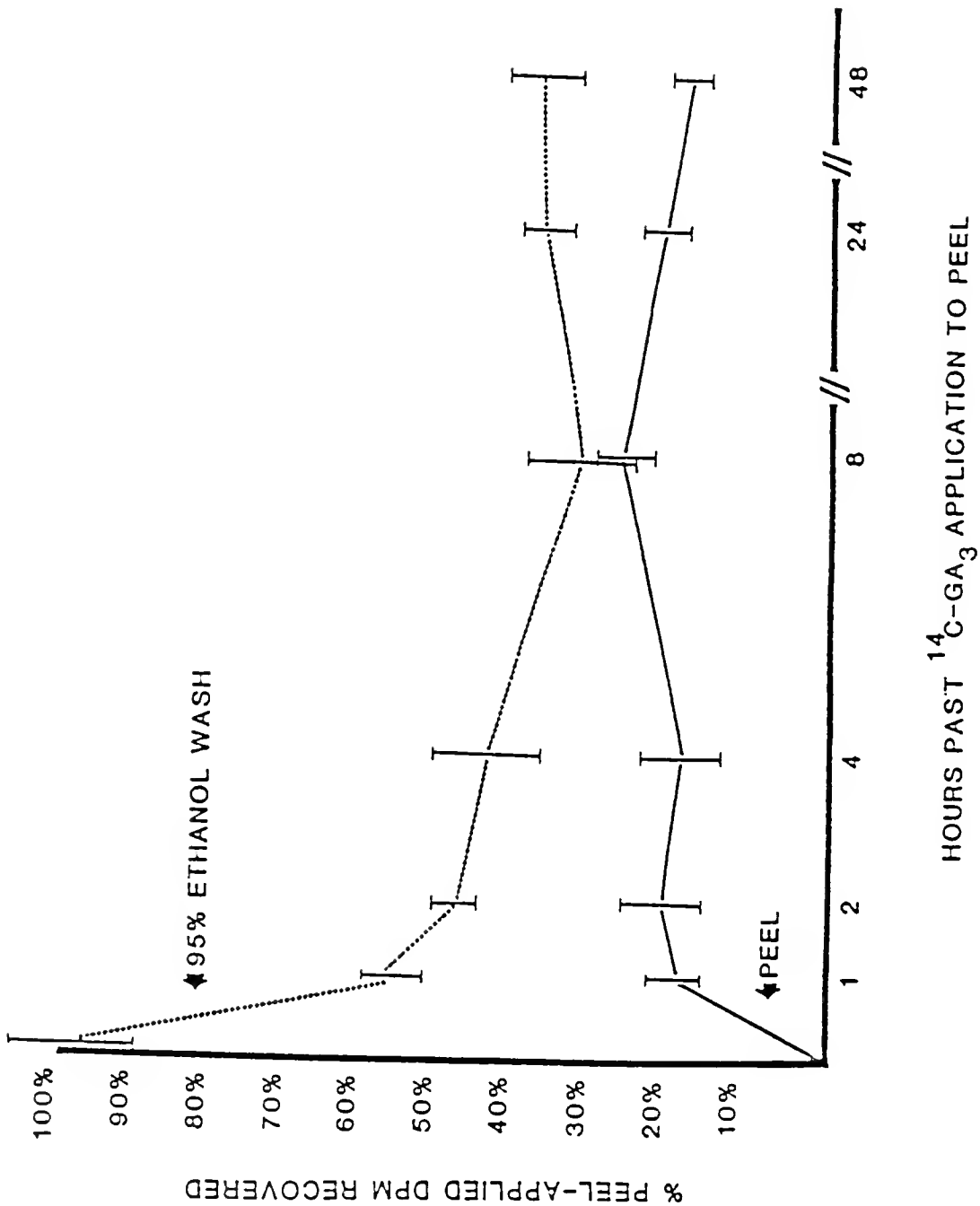

% PEEL-APPLIED DPM RECOVERED
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
95% EtOH WASH
PEEL
TWIG
LEAF
0
1
2
4
8
1
2
4
8
HOURS
DAYS
WEEKS
TIME PAST ^{14}C-GA_3 APPLICATION TO PEEL

Fig. 4-4. Recovery of peel-applied radioactivity from detached grapefruit (albedo and flavedo combined). Means of nine replications per time.


% PEEL-APPLIED DPM RECOVERED
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
95% ETHANOL WASH
PEEL
1
2
4
8
24
48
HOURS PAST ^{14}C-GA_3 APPLICATION TO PEEL

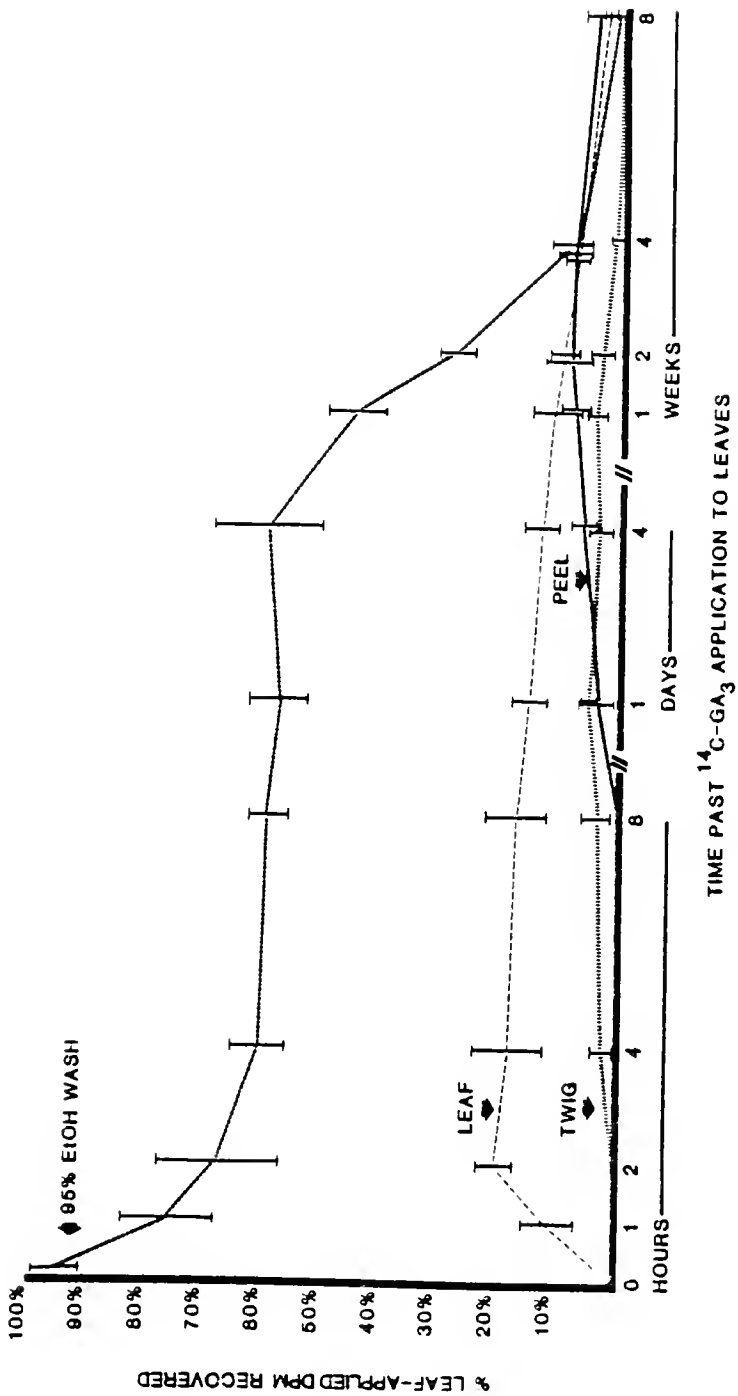
Uptake, Translocation, and
Persistence of Leaf-Applied ^{14}C-GA_3

Leaves began absorbing leaf-applied ^{14}C-GA_3 immediately, reaching a maximum level within 2 hours (Fig. 4-5). The amount of label decreased steadily over the next 8 weeks. Radioactivity was present in twigs within 2 hours and persisted over 4 weeks. Radioactivity was extracted from the peel within 8 hours. It reached a maximum at 2 weeks and then diminished slowly over 8 weeks. Residual ^{14}C-GA_3 on the leaf surface decreased sharply for the first 2 hours and steadily thereafter. No radioactivity was recovered from seeds or pulp.

Both leaves and peel absorbed ^{14}C-GA_3 within 1 hour, but peel absorbed more in a shorter time and accumulated a greater amount whether the ^{14}C-GA_3 was peel- or leaf-applied. This apparently greater uptake may be the result of better uptake by the peel or less translocation away from the peel than from the leaves. The greater accumulation may be the result of photosynthate translocation from leaf to peel.

The recovery of radioactivity from peel tissues reported here is eightfold that previously reported for citrus peel application (39). The total radioactivity recovered within 8 hours was 73.7% of peel-applied and 80.2% of leaf-applied radioactivity. Radioactivity recovered from detached fruit was less: a total of 58.8% 8 hours after application. Possible explanations for these losses include incomplete extraction, metabolism to other compounds or oxidation to CO_2, and translocation to other tissues. The first possibility cannot account for all the losses; the extraction procedure used had a 91.2% efficiency, determined using a ^{14}C-GA_3 standard (Fig. 4-1). Davies and Rappaport (22) demonstrated that GA_3 is rapidly metabolized in higher plants, which could

Fig. 4-5. Recovery of leaf-applied radioactivity from grapefruit leaves, peel (albedo and flavedo combined), and twigs. Means of nine replications per time.


% LEAF-APPLIED DPM RECOVERED
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
95% EtOH WASH
LEAF
TWIG
PEEL
0
1
2
4
8
HOURS
1
2
4
8
DAYS
1
2
4
8
WEEKS
TIME PAST ^{14}C-GA_3 APPLICATION TO LEAVES

account for some losses; however, the most likely cause of these losses is translocation to tissues not sampled. Translocation of radioactivity begins within 2 hours and is not limited to fruit, subtending leaves, and twigs. Low levels of radioactivity were measured in twigs proximal to sampled leaves within 2 to 4 hours of application (data not shown). However, translocation cannot account for the poor recovery levels in detached fruit. Possibly detached fruit metabolize absorbed GA_3 more rapidly; Goldschmidt and Galili (37) reported that the biological effects of GA_3 on detached 'Shamouti' oranges were less marked and persistent in detached than in attached fruit.

The translocation of radioactivity reported here agrees with the report by Goldschmidt and Eilati (37) suggesting diffusive movement of GA_3 in the flavedo. However, they observed only flavedo, which lacks vascular tissue. Vascular tissue does exist in the albedo, where radioactivity was recovered in the present study. Therefore, vascular transport of absorbed radioactivity once it diffuses from the flavedo to the albedo would be a reasonable assumption. Translocation of gibberellins via the vascular system has also been reported to occur in other higher plants (13,66)

The long persistence of radioactivity reported here is supported by an earlier study of Goldschmidt and Galili (39). However, they reported only 2% recovery of peel-applied ^{14}C-GA_3 after 24 hours and less than 1% after 100 days, compared with the 16.7% at 24 hours and 6.7% at 56 days reported here.

The uptake, translocation, and persistence of ^{14}C-GA_3 by citrus peel determined here are consistent with the observed field effects of GA_3 on grapefruit. Preharvest applications of GA_3 delay color

development and loss of peel firmness, but do not affect seed sprouting or juice quality (2,25,35,59). These effects are evident within 1 month and may persist 6 months. Data here demonstrate that leaf or peel application of ^{14}C-GA_3 results in rapid accumulation of radioactivity in the peel (within 24 hours), but not in the seeds or juice, even after 8 weeks. These studies also demonstrate persistence of measurable radioactivity in peel tissues after 8 weeks. It is not known if these levels are physiologically active or what the radioactive compound is, but the sites of accumulation are consistent with the effects produced by spray treatments of unlabeled GA_3 at the same concentration.

Separation of Extracted Radioactivity by High-Performance Liquid Chromatography

Metabolism of applied ^{14}C-GA_3 in both attached and detached fruit was quite similar. However, the time course of the former was longer. Therefore, only the metabolism data for the attached fruit will be discussed.

Koshioka et al. (60) reported a "double-peaking" phenomenon with the use of a reversed-phase HPLC C18 column for gibberellin separations. They found that the degree of sample purification caused retention time shifts and recommended that C18 columns be used only for highly purified samples. The same problem arose with the similar reversed-phase u-Bondapak phenyl column (Waters Associates) used in this study. The presence of leaf, flavedo, or albedo extract in samples consistently reduced retention times by 2 minutes. This was a problem because suspected metabolites of GA_3 have been demonstrated to elute within 1 to 4 minutes earlier than GA_3 on reversed-phase columns (60). The possibility of mistaking less purified samples for metabolites was

solved by establishing the retention time of ^{14}C-GA_3 in the presence of albedo, flavedo, and leaf extracts.

Radioactivity recovered from flavedo, albedo, and leaves consistently eluted in the same fractions (Fig. 4-6). Radioactivity in fractions 12-15 consistently peaked in fraction 13, co-chromatographing with the ^{14}C-GA_3 standard, and radioactivity in fractions 4-7 consistently peaked in fraction 6. The only exception was albedo extracts, which eluted almost as much radioactivity in fraction 14 as in 13. In all tissues the ratio of the two fractions changed rapidly over the first 4 days after application, then remained approximately stable over the next 8 weeks (Fig. 4-7). Radioactivity recovered in fractions 4-7 increased from 1.2% in a total of 30.2% applied activity recovered at 1 hr to 8.1% of 16.9% applied activity recovered at 4 days. Therefore, the percentage of radioactivity recovered in fractions 12-15 decreased from over 96% of the recovered activity at 1 hour to 52% at 4 days, while the percentage of activity recovered in fractions 4-7 increased correspondingly. This pattern is consistent with reports of rapid metabolism of absorbed gibberellins in other higher plants (14,22). As plant tissues metabolize ^{14}C-GA_3, the 12-15 peak diminishes and the 4-7 peak increases. The ^{14}C-GA_3 is metabolized over time until a relatively constant ratio between the two fractions is attained. Alternately, ^{14}C-GA_3 may be metabolized by plant tissues until it can be compartmentalized, possibly in vacuoles (76). Once this occurs, the ^{14}C-GA_3 is slowly released as needed and subsequently degraded, maintaining a fairly constant ratio of ^{14}C-GA_3 and its metabolites (43). Studies using radiolabeled GA_3 applied to 'Shamouti' and 'Valencia' orange peel support these possibilities (38,39). In both cases an ethylacetate or acid

Fig. 4-6. Chromatogram of radioactivity extracted from peel 1 hour after application of ^{14}C-GA_3 to peel. Elution of radioactivity from analytical reversed-phase HPLC system with u-Bondapak phenyl column. Linear gradient of 95% ethanol (0-50%) in 0.2% NH_4Ac at pH 5.6, run in 50 minutes at 1.5 ml per minute. Fractions collected every minute. Chromatogram is the average of nine replications.

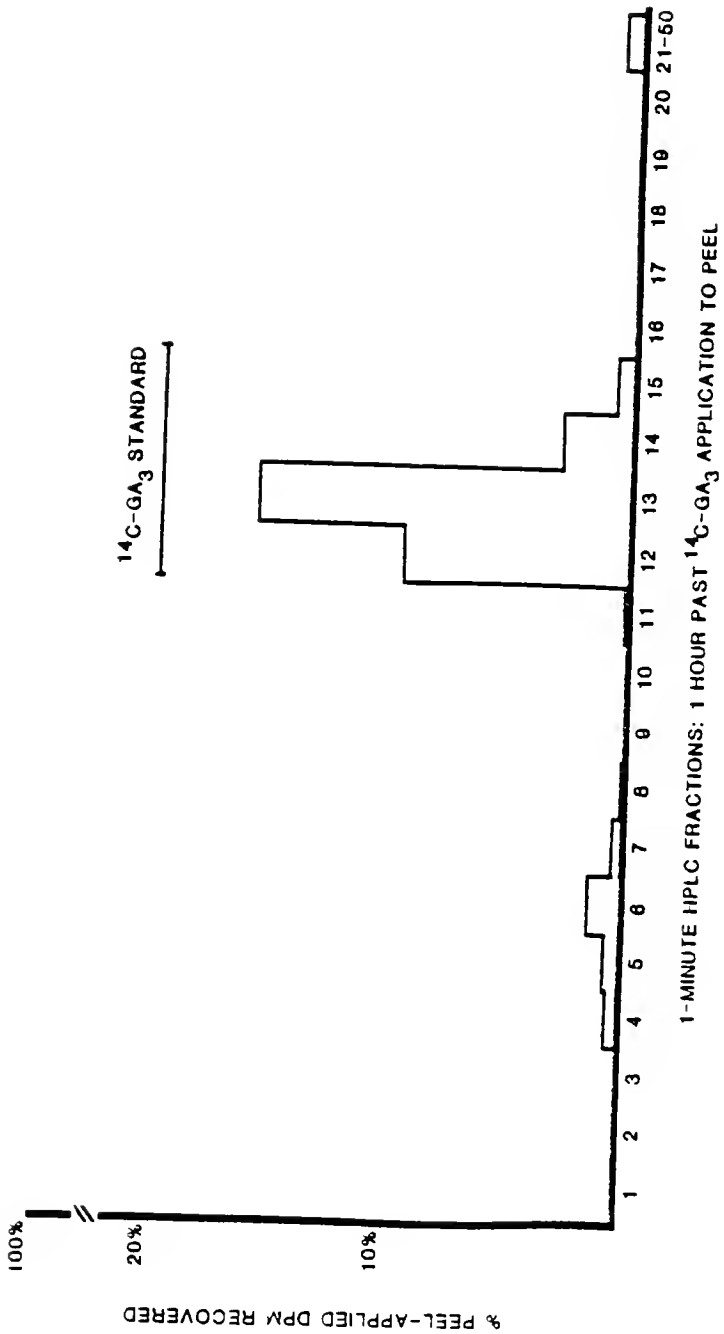
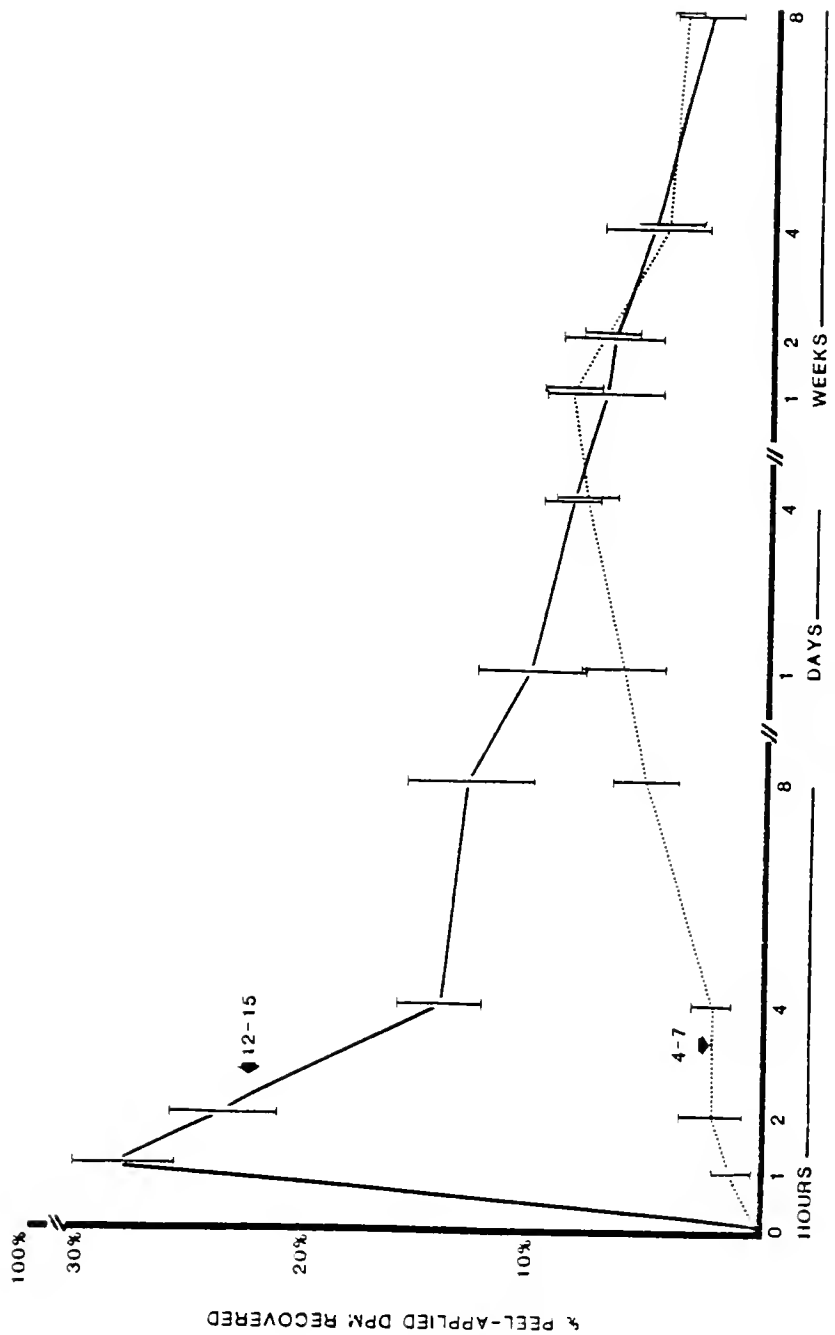

% PEEL-APPLIED DPM RECOVERED
100%
20%
10%
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21-50
1-MINUTE HPLC FRACTIONS: 1 HOUR PAST ^{14}C-GA_3 APPLICATION TO PEEL
^{14}C-GA_3 STANDARD

Fig. 4-7. Recovery of peel-applied radioactivity from peel (albedo and flavedo combined) in HPLC fractions 4-7 and 12-15. Each point is an average of nine values. Standard deviations are indicated by bars.


% PEEL-APPLIED DPM RECOVERED
100%
30%
20%
10%
12-15
4-7
0
1
2
4
8
HOURS
1
4
DAYS
1
2
4
8
WEEKS
TIME PAST ^{14}C-GA_3 APPLICATION TO PEEL

diethyl ether fraction and a water-soluble fraction were extracted, which were tentatively identified as GA_3-like and metabolite fractions, respectively.

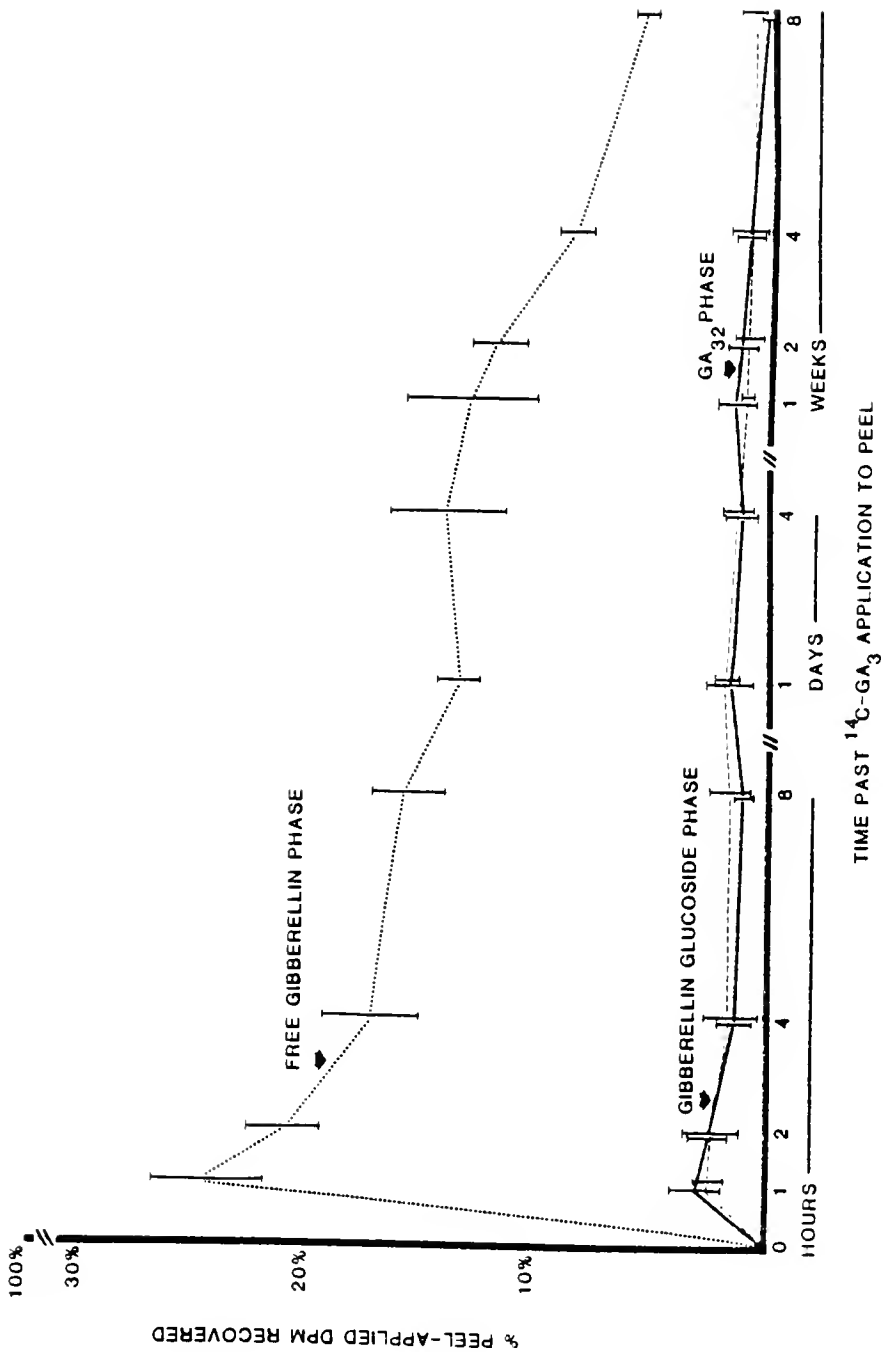
B-D-glucosidase Hydrolysis of Radioactive Fractions

The C13 and C3 glucose ethers are transport and storage metabolites of GA_3 in higher plants; they are biologically inactive relative to GA_3 (7). These metabolites have slightly lower retention times than GA_3 on reversed-phase columns (60). Hydrolysis of the glucose moiety from the GA_3 increases the retention time of this fraction. Hydrolysis of fractions 12-15 and fractions 4-7, however, produces no change in retention times, indicating that the radioactivity recovered in fractions 4-7 and 12-15 is probably not the C3 or C13 ethers of ^{14}C-GA_3. This suggests that grapefruit peel and leaves do not conjugate GA_3, but metabolize it.

N-butanol of Radioactive Fractions

Partitioning gibberellins with N-butanol is a method of separating free gibberellins from their esters and ethers. The results of partitioning fractions 4-7 were inconclusive (data not shown); all recovered radioactivity partitioned into the polar metabolite phase. Partitioning fractions 12-15 gave 83.3% in the free gibberellin phase, 8.5% in the polar metabolite phase, and 7.9% in the glucoside phase (Fig. 4-8). When these phases were combined and rechromatographed, radioactivity recovered in the polar metabolite and GA glucoside phases was insignificant. The radioactivity in the free gibberellin phase co-chromatographed with the ^{14}C-GA_3 standard. The rather constant

Fig. 4-8. Recovery of peel-applied radioactivity in free gibberellin, gibberellin glucoside, and polar metabolite phases following N-butanol partitioning of fractions 12-15 of peel tissues (albedo and flavedo values combined). Means are an average of nine replications. Standard deviations are indicated by bars.


% PEEL-APPLIED DPM RECOVERED
100%
30%
20%
10%
FREE GIBBERELLIN PHASE
GIBBERELLIN GLUCOSIDE PHASE
GA32 PHASE
0
1
2
4
8
1
2
4
8
HOURS
DAYS
WEEKS
TIME PAST 14C-GA3 APPLICATION TO PEEL

percentage of radioactivity that partitioned into the polar metabolite and GA glucoside phases suggests that the radioactivity recovered in these phases may be the result of inefficient partitioning.

These results suggest that the ^{14}C-GA_3 accumulated in grapefruit peel remains in the applied form, possibly sequestered in the vacuoles, and is slowly metabolized to a more polar form.

Conclusions

These uptake, translocation, persistence, and metabolism studies partially clarify the fate of GA_3 applied to grapefruit peel and leaves. Absorption of ^{14}C-GA_3 by leaves and peel began within 1 hour of application and continued for 8 hours. Peel absorbed more radioactivity at a faster rate than leaves. Translocation of radioactivity from leaves to peel and the reverse began in 4 to 8 hours and continued for 4 weeks. No radioactivity was recovered from juice or seeds. Radioactivity persisted in albedo, flavedo, and leaves for 8 weeks with the highest accumulations in peel tissues. Separation of ^{14}C-GA_3 metabolites by reversed-phase HPLC produced two peaks of radioactivity. Analysis of these two peaks by B-D-glucosidase hydrolysis, N-butanol partitioning, and co-chromatography with ^{14}C-GA_3 standards suggested that one was ^{14}C-GA_3 and the other, metabolites.

CHAPTER V

CONCLUSIONS

There were three objectives to this dissertation. The first was to determine if decreased irrigation affected the ability of a preharvest GA_3 and 2,4-D colorbreak spray to extend the harvest season of 'Marsh' white grapefruit. The second was to compare preharvest and postharvest, and combined preharvest and postharvest, treatments with GA_3 and 2,4-D for ability to extend the storage life of grapefruit. The third objective was to study the translocation and metabolism of ^{14}C-GA_3 in grapefruit leaves and fruit.

Decreased late-season irrigation did not affect the ability of preharvest GA_3 and 2,4-D sprays to extend the harvest season of 'Marsh' grapefruit in Florida. The GA_3 delayed overripe color development and loss of peel firmness without affecting internal quality. The 2,4-D decreased late-season fruit drop. When the GA_3 and 2,4-D sprays were applied 6 weeks prior to a mild freeze, they decreased postfreeze fruit drop.

A preharvest GA_3 and 2,4-D spray or postharvest GA_3 and 2,4-D dip maintained peel quality in storage equally well for fruit harvested through mid-March. However, preharvest sprays had the added advantage of extending the harvest season and providing postfreeze fruit-drop protection. Applying both preharvest spray and postharvest dip maintained fruit quality better only if fruit was harvested in mid-May.

Both preharvest and postharvest treatments produced the same effects: external peel quality was maintained while internal quality was unaffected. All treatments decreased decay in storage equally well on all harvest dates.

Peel and leaves began absorbing ^{14}C-GA_3 within 1 hour of application and continued to do so for 8 hours. Leaves absorbed ^{14}C-GA_3 more slowly than peel. Within 2-4 hours of application, radioactivity was translocated from peel to leaves and the reverse. Radioactivity, whether applied or translocated there, persisted in leaves and peel tissue for 8 weeks. However, accumulation of radioactivity was always greater in peel than in leaves. No radioactivity was recovered from seeds or juice. Separation of peel-extracted metabolites by reversed-phase HPLC produced two peaks of radioactivity. Analysis of these peaks suggested that one was ^{14}C-GA_3 and the other, a polar metabolite.

The results of these three studies are consistent with one another. Applied ^{14}C-GA_3 was readily absorbed, accumulated in peel but not internal tissues, and persisted for at least 8 weeks. The spray and dip GA_3 treatments produced changes in peel, but not internal, quality that were measurable within 6 weeks and persisted as long as 5 months. Therefore, radioactivity accumulated and persisted in the same tissues in which GA_3 sprays and dips produced their effects. It is not known if the radioactivity extracted from peel tissues is the active form of GA_3 or if the levels measured are physiologically active. However, the steady decline of the extracted radioactivity and its possible degradation are consistent with the decreasing effects of GA_3 on peel quality over time.

The last remaining question is, How does GA_3 produce its effects? Does absorbed GA_3 or its metabolites initiate a physiological process, then undergo degradation? Or are the effects produced as long as GA_3 or active metabolites persist? Evidence presented in these studies supports both possibilities, but the latter more strongly. The effects of GA_3 on peel quality were more marked when it was applied earlier; preharvest applications produced greater effects than postharvest applications at lower concentrations. However, this may simply be the result of delaying a process, peel senescence, earlier in its development. The ability of GA_3 to produce an effect, no matter when applied, and the persistence of ^{14}C-GA_3 argue for the latter possibility. This possibility is supported by the fact that peel senescence in citrus is associated with a decrease in endogenous gibberellins. Therefore, the data in these studies suggest that applied GA_3 delays grapefruit peel senescence as long as the absorbed GA_3 or its metabolites persist in physiologically active levels.

APPENDIX

GROWTH REGULATOR AND NUTRITIONAL EFFECTS ON GRAPEFRUIT COLOR AND STORAGE QUALITY

Literature Review

Effects of Nitrogen and Gibberellins on External Grapefruit Quality

An increase in tree nitrogen within the range for high-level production has what are usually regarded as adverse effects on the external quality of citrus (28). In most cases nitrogen produces a coarser, thicker peel and a firmer albedo, and delays color development. These effects are well documented on navel, 'Valencia', pineapple, and 'Hamlin' oranges and grapefruit (4,5,26,28,55,56,64,71,80, 81,83,84,95). High levels of nitrogen in grapefruit produced delays in color development and firmer peels in Arizona (28) and Florida (96) grapefruit. Results were similar for foliar and soil-applied nitrogen (28). The effects of nitrogen on external peel quality are similar to those produced by GA_3 (see Chapter II). The combination of nitrogen and GA_3 in a foliar spray produces greater effects than either individually; Monselise et al. (71) and Embleton et al. (26) demonstrated that the addition of potassium nitrate or ammonium phosphate to GA_3 sprays reduced the incidence of creasing and delayed orange color development in 'Valencia' oranges better than the nutritional or GA_3 treatment alone. Monselise suggested that nitrogen application enhances the production of endogenous gibberellins.

Effects of Nitrogen and Gibberellins on Internal Grapefruit Quality

The effects of nitrogen on internal quality are inconclusive and contradictory (27,28). Nitrogen applications to grapefruit have been reported to reduce the percentage of juice and incease the acidity and total soluble solids (TSS) without affecting the TSS/acid ratio (26,28), to decrease the TSS and the TSS/acid ratio (28), to have small, inconsisten effects on these factors (4,83,84), and to decrease the percentage of juice up to 2.6% of leaf dry weight of nitrogen and decrease it less thereafter (27). The inconsistency of these effects may be a result of when the nitrogen is applied (28). There are no reports that soil or foliar applications of nitrogen decrease seed sprouting or section drying in grapefruit. The effects of GA_3 on internal fruit quality were covered in Chapter II; generally, effects are small and inconsistent. There are no reports on the effects of combined nutritional and gibberellin treatments on grapefruit internal quality.

Little work has been done on the effects of nutritional sprays, alone or combined with gibberellins, on grapefruit quality in Florida. The objective of this study was to compare nitrogen and gibberellin colorbreak sprays for their ability to delay color change and seed sprouting of tree-stored and cold-storage fruit under Florida conditions. Juice quality and decay in storage were also observed.

Materials and Methods

Thirty mature 'Marsh' grapefruit (Citrus paradisi Macf.) trees on rough lemon (C. jambhiri Lush.) rootstock were selected from a grove near Lucerne Park, Florida. Trees were planted at 4.5×9.1 m spacing. Cultural practices and soil type (deep ridge sand) were typical of local commercial groves.

Completely randomized single-tree plots were used with four treatments and six replications of each. Treatments were (1) KNO_3 (2%) + NH_4NO_3 (2%); (2) GA_{4+7} (20 ppm); (3) GA_3 (20 ppm); and (4) KNO_3 (2%) + NH_4NO_3 (2%) + GA_3 (20 ppm). All treatment and control water spray contained X-77 (0.025%). Pro-Gibb® was the GA_3 source, Promalin® was the GA_{4+7} source, and both nutritional sprays contained approximately 35% nitrogen. Approximately 50 liters of dilute spray were applied per tree on December 5, 1979.

Twenty fruit per tree were evaluated prior to treatment and bimonthly January through June. Color, juice quality, and seed sprouting were tested by use of the Hunter Color Difference Meter (Fairfax, VA), automated systems analysis (23), and observation, respectively.

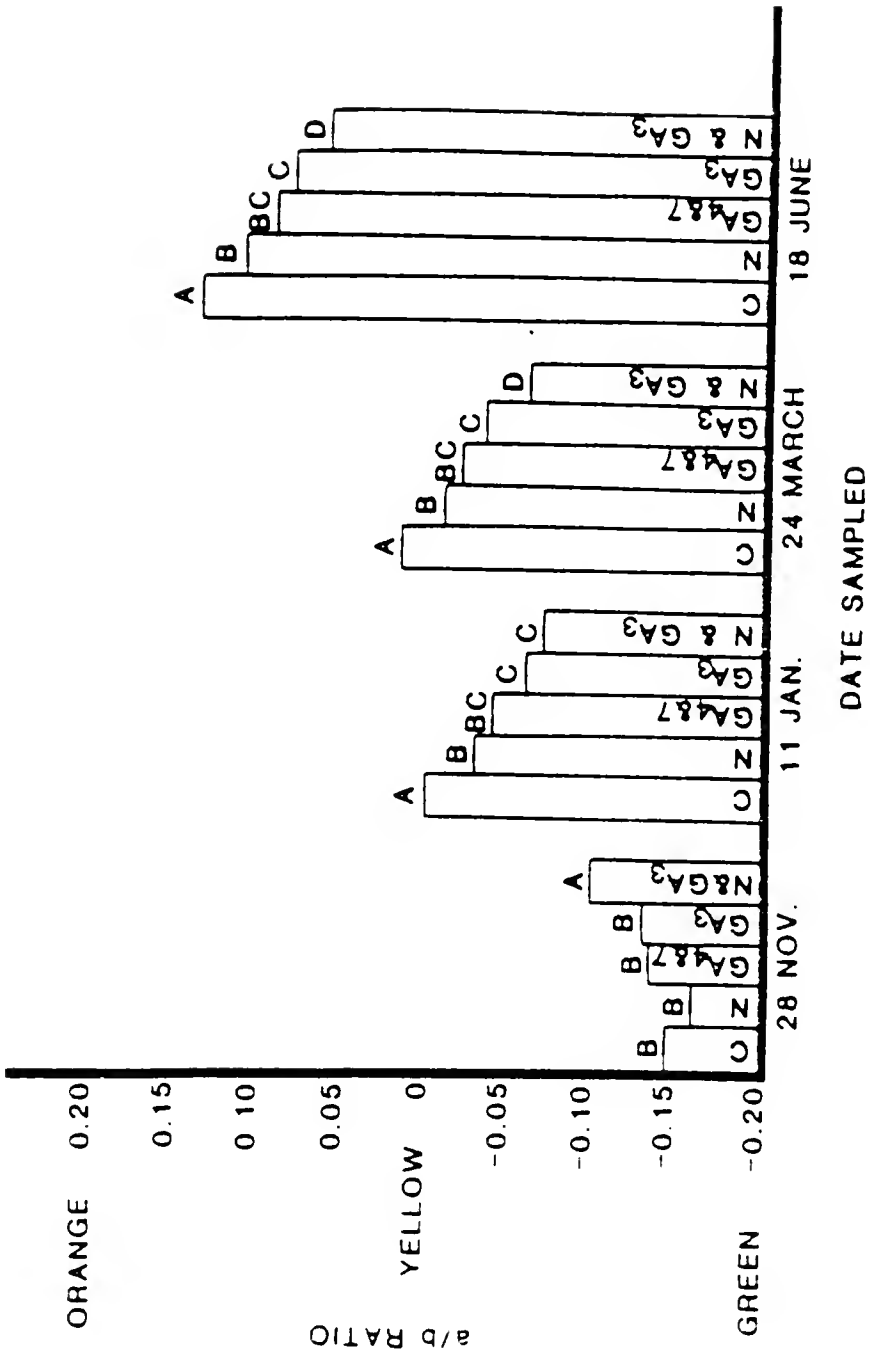
Eighty fruit per tree were harvested in March, washed, treated with thiabendazole (1000 ppm) as a fungicide, waxed, packed into 20-kg cartons at 30 fruit per carton, and stored at 15.5°C and 96% relative humidity for 14 weeks. Juice analysis, seed sprouting, and color evaluation of samples of 20 fruit per replication were done before and after storage. Surface decay was evaluated weekly.

Results and Discussion

Peel Color of Tree-Stored Fruit

All treatments delayed peel color change compared with controls (C) (Fig. A-1). Combined nutritional (N) and GA_3 treatment produced the best results in March and June. This supports previous evidence that combined nitrogen nutritional and GA_3 sprays delayed color change in 'Valencia' oranges better than either alone (19,26). GA_{4+7} and GA_3 alone were equally effective.

Fig. A-1. Treatment effects on color of tree-stored grapefruit. Bars with different letters are significant at P = <0.01. Bars are averages of 120 values.


a/b RATIO
ORANGE
YELLOW
GREEN
0.20
0.15
0 10
0.05
0
-0.05
-0.10
-0.15
-0.20
28 NOV.
11 JAN.
24 MARCH
18 JUNE
DATE SAMPLED
C
N
GA4&7
GA3
N & GA3
A
B
BC
C
D

Internal Quality of Tree-Stored Fruit

There were no significant differences in internal quality prior to treatment, and treatments had no significant effects on total soluble solids (TSS), percentage of acid, or seed sprouting (data not shown). Compared with controls, all treatments increased the percentage of juice by January and by March; only trees treated with GA_3, alone or combined with nutritional treatment, had increased juice content. By June only GA_3-treated fruit had significantly higher juice content (Table A-1). There were no differences among treatments. Only combined nutritional and GA_3 treatment produced a significantly higher ratio than controls on all dates. However, there was little difference among treatments. These results contradict previous results showing that nitrogen sprays decreased the TSS/acid ratios in 'Valencia' oranges by increasing the percentage of acid (19,26). Percentage of acid of all treated fruit was not significantly lower than that of controls (data not shown). Reports of inconsistent effects of growth regulators on internal quality of grapefruit are common in the literature (2,25,35,59). The incidence of seed sprouting in treated fruit was equal to that of controls, remaining below 3% through March and increasing to 15-19% in June (data not shown). This disagrees with one report (2), which showed that GA_3 decreased seed sprouting in grapefruit, and agrees with another (1). Perhaps seed sprouting is a function of harvest date. The dissenting report (2) had an earlier harvest date than this study and the other report (1).

Table A-1. Effects on internal quality of tree-stored grapefruit.

	Date Sampled					
	January 11		March 24		June 18	
	% Acid	TSS/Acid	% Juice	TSS/Acid	% Acid	TSS/Acid
	**	*	*	**	†	**
Control	53A	8.2A	53A	8.8AB	51A	10.9A
KNO_3 and NH_4NO_3	55B	8.6AB	54AB	8.5A	52AB	10.9A
GA_{4+7}	55B	8.8AB	54AB	9.5BC	56AB	11.7B
GA_3	56B	8.4A	55B	9.3ABC	57B	11.3A
KNO_3 and NH_4NO_3 and GA_3	55B	9.2B	55B	10.2C	53AB	13.3B

Note: Column means with different levels are significant as follows: **, P = <0.01; *, P = <0.50; †, P = <0.10. N = 120.

Peel Color of Cold-Storage Fruit

All treatments delayed peel color change in cold-storage fruit with little difference among treatments (Fig. A-2). Tree-stored fruit receiving the same treatments changed color more rapidly during this time period with one exception. Untreated control fruit in cold storage changed color twice as rapidly as tree-stored control fruit. This suggests that all the treatments are particularly effective in delaying postharvest acceleration of color change (37) as well as delaying color change preharvest.

Internal Quality of Cold-Storage Fruit

There were no significant differences in internal quality prior to storage or in percentage of juice or seed sprouting after storage (data not shown). The TSS were increased in fruit receiving combined growth regulator and nutritional treatment, and the percentage of acid was decreased in all treated fruit. All treated fruit therefore had higher TSS/percentage of acid ratios than controls (Table A-2). Seed sprouting was not different from that in controls, remaining below 4% for all treatments. Correspondingly treated tree-stored fruit had a much higher incidence of sprouting, 15-19%, which was perhaps due to higher temperatures in the grove than in cold storage and to a later harvest date (1).

Decay During Cold Storage

All growth regulator treatments significantly decreased decay in storage, but GA_{4+7} and combined GA_3 and nutritional treatment were the most effective (Fig. A-3).

Fig. A-2. Treatment effect on color during cold storage. Bars with different subscripts and superscripts (color at harvest an after storage) are significant at P = <0.01. Bars are an average of 120 values.

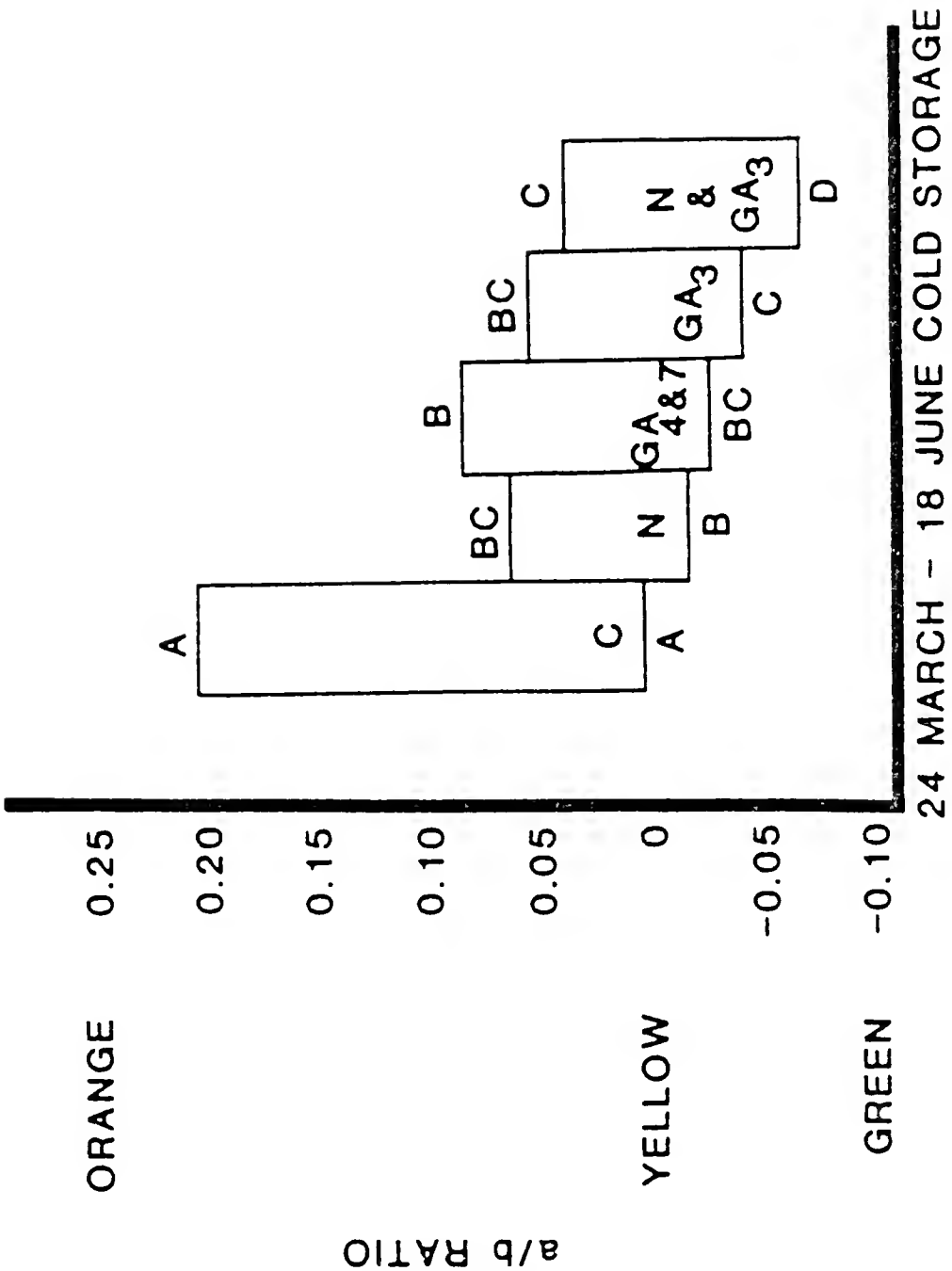
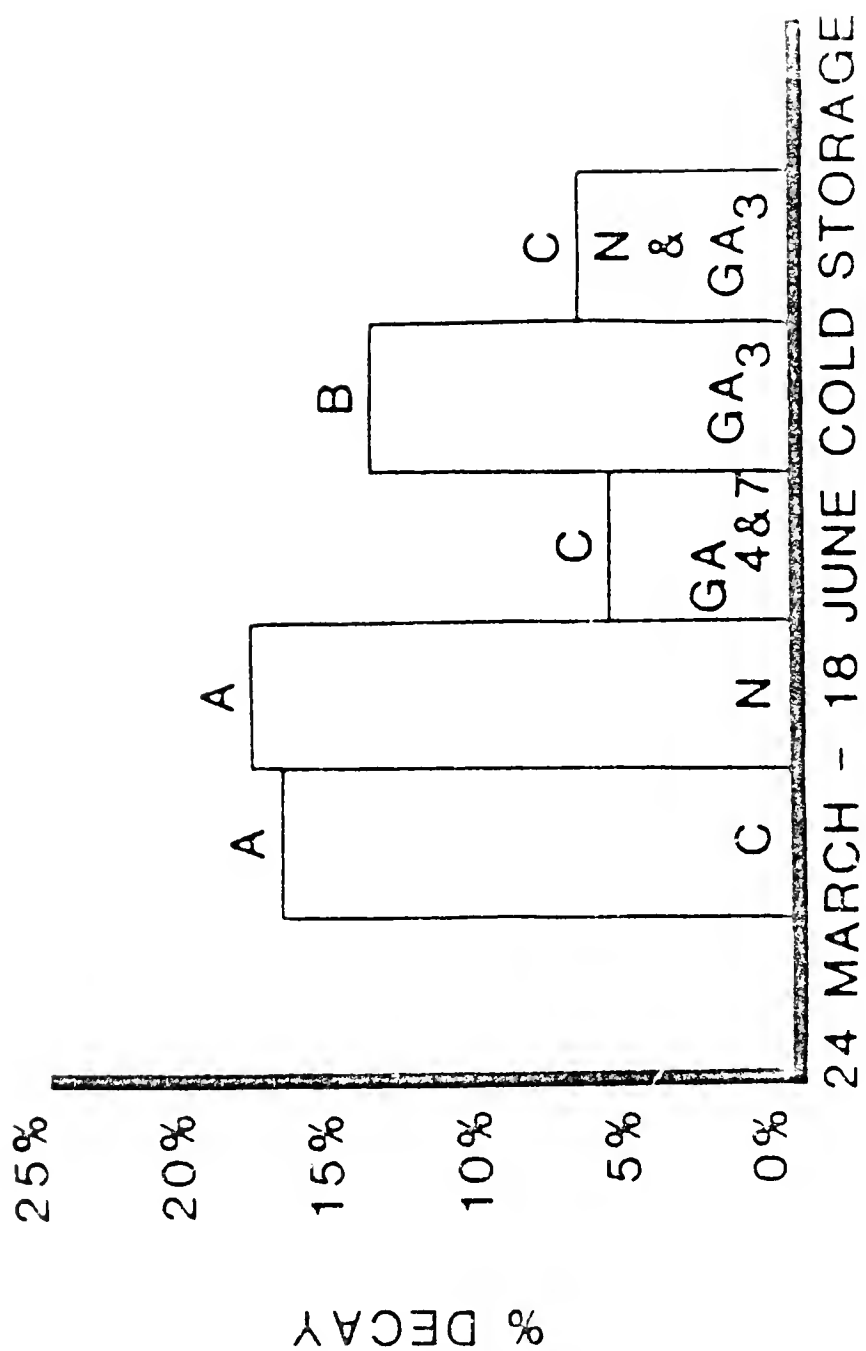

a/b RATIO
ORANGE
YELLOW
GREEN
0.25
0.20
0.15
0.10
0.05
0
-0.05
-0.10
24 MARCH - 18 JUNE COLD STORAGE
A
C
BC
N
A
B
B
GA4&7
BC
BC
GA3
C
C
N & GA3
D

Table A-2. Effects on internal quality of cold-storage grapefruit.

	Date Sampled: June 18		
	TSS	% Acid	TSS/Acid
Control	9.1B	0.9B	10.4A
KNO_3 and NH_4NO_3	8.6A	0.8A	11.6B
GA_{4+7}	9.4B	0.8A	11.4B
GA_3	9.1B	0.8A	11.2B
KNO_3 and NH_4NO_3 and GA_3	9.8C	0.8C	12.9C

Note: Column means with different letters are significant at P = <0.01. N = 120.

Fig. A-3. Treatment effect on grapefruit decay during cold storage. Bars with different letters are significant at P = <0.01. Bars are an average of 120 values.


% DECAY
25%
20%
15%
10%
5%
0%
24 MARCH - 18 JUNE COLD STORAGE
A
A
C
N
C
GA 4&7
B
GA3
C
N & GA3

Conclusions

The additive effects of combined nutritional and GA_3 spray were most effective in delaying color change of tree-stored fruit March to June. Only GA_{4+7} and GA_3 treatments were less effective than combined treatment and they were equal to each other. Only GA_3 was more effective than the nutritional treatment. All four treatments were equally effective in delaying color change of fruit in cold storage. All four treatments produced inconsistent changes on juice quality and had no effect on seed sprouting. These results support previous reports (1,2) that indicate that seed sprouting may be a function of temperature and harvest date and is not affected by applied growth regulators. Decay in storage was reduced by combined GA_3 and nutritional treatment, GA_3 alone, and GA_{4+7}. These results support the use of combined gibberellin and nutritional sprays to delay color change of tree-stored and cold-storage grapefruit and to delay decay in cold storage.

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